A STUDY OF THE NATURAL IMMUNITY REACTIONS OF ANIMAL SERA WITH REFERENCE TO NUTRITIONAL VARIATION, INCLUDING A STATISTICAL SURVEY OF THE RESULTS.

A THESIS

BY

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CONTENTS.

INTRODUCTION.

It has long been recognised that nutritional factors are concerned in the natural resistance of the animal body to infective disease and recent evidence (Green & Mellanby 1929) that vitamin A deficiency predisposes to infection by commensal bacteria emphasises the importance of immunological studies in relation to nutrition. The literature of the subject has been reviewed recently by Ledingham (1931). Some workers have approached the study of immunity in relation to nutrition by experiments on laboratory animals with induced infection, (Findlay (1923), Cramer & Kingsbury (1924), Webster & Pritchett (1924), Arkwright & Zilva (1924), Lassen (1930), Topley, Greenwood & Wilson (1931)), and experimental work on such lines undoubtedly affords direct evidence on the question. Little has been done, however, on the influence of dietary factors on the activity of the antibody-forming mechanism as estimated by serum reactions. In 1929 the writer was given the opportunity of investigating, at the Rowett Institute, Aberdeen, this aspect of the subject, on sheep under experiment for nutritional studies primarily of a physiological or biochemical nature, and the thesis submitted records the results of this work. Investigations on this subject have been carried out in recent years and the literature is briefly reviewed below.

Smith & Wason (1923) examined the complementing power, bactericidal and bacteriotropic activity of the serum of animals kept on a rickets-producing diet, low in phosphorus and vitamin A, as compared with those of normal contro animals. No difference in complementing activity was found, but in both the other reactions the control animals showed markedly higher values than the animals on the experimental diet. This was particularly the case with the bactericidal/

bactericidal reaction. Zilva (1919) fed rats and guinea-pigs on diets deficient in several elements, vaccinating them with B. typhosus. The animals on a phosphorus deficient diet, instead of developing an agglutinin increase, actually showed a fall in the agglutinin value; in the groups receiving diets low in calcium and phosphorus and those kept for a prolonged time on a generally restricted diet there was a diminished agglutinin response compared with the controls. Vasile (1929) compared the complementing power of the serum of infants suffering from nutritional disturbance with that of normal infants and stated that it was much lower. According to Osborn diets poor in vitamin A tended to lower the serum complement of rats below that of normally fed controls. Lawrynowicz (1931) found a reduction in the number and phagocytic properties of leucocytes in animals during avitaminosis but no significant change in complement. These findings seem to suggest that vitamins are concerned in the bodily mechanisms of defence. There is, however, evidence which suggests that other dietary factors play some role in this phenomenon. Thus it has been shown (Weiss, Sümegi & Benkovico, 1928) that when the pH of the blood was altered by administration of alkali, the haemolysin titre was only half as great as in animals in which the pH was lowered by administration of acid. It is true that this difference was only transient - appearing in the first week of treatment but not in the second and third weeks. Scholtz (1931) reported that injections of magnesium sulphate increased the bactericidal power of rabbit's blood towards Staph. aureus, while calcium gluconate had no effect. The findings of Tunnicliff (1931), on the other hand, were that when this latter substance was given to a man by three routes in succession - intravenous, intramuscular, and oral - the phagocytic action of the leucocytes

was/

was increased above the level prevailing before and after treatment. She also showed that intravenous injection in rabbits of calcium gluconate, calcium chloride, sodium salicylate, sodium iodide, dextrose, or neosalvarsan was followed by a rapid rise in phagocytic power of the leucocytes, returning to normal in three to four hours. Ledingham (1931), on the other hand, in summing up the effect of nutrition on resistance to disease, comes to the conclusion that there is little evidence that nutritional defects disturb the antibody-forming mechanism.

Recently the natural immunity reactions of animal sera have been systematically and extensively studied by Mackie and Finkelstein (1930, 1931 & 1932) and by Gibson (1931). They investigated the occurrence and nature of complement fixation by interaction of normal serum and bacterial emulsions, and of the bactericidal and agglutinating powers of normal serum. They studied the serum reactions of different animal species against bacterial organisms varying in species and strain. From the results of these researches they brought forward evidence to show that all animals possess these natural serum properties varying in degree according to species, individual, and the organism used. Further, these properties are due to natural antibody-like principles, which may act with or without complement, as in the case of the bactericidins against Gram-negative organisms and the agglutinins respectively. They are specific for one organism, except in the case of the β-lysin for Gram-positive organisms, which, however, seems to stand in a category by itself. The various animal species may be grouped in order of the activity of their bactericidins, agglutinins, etc. though the order is not the same among the different types of reactions, and a marked variation occurs among individual animals/

animals.

The nutrition experiments on sheep at the Rowett Institute presented an opportunity of ascertaining whether the natural immunity reactions are influenced by varying nutritional states and thus obtaining further information regarding the effect of dietary factors on the antibody-forming mechanism. It is, of course, recognised that such natural antibacterial properties may not be direct indices of actual resistance and that they do not constitute a complete mechanism of defence of the animal organism, but, on the other hand, it is only reasonable to suppose that they play an important contributory part in natural immunity. At the outset it should be explained that the work done so far has been essentially exploratory. It was hoped that, in the course of the investigation, certain serum reactions might be found to correspond quantitat-:ively with the nutritional state of the animals and that such reactions might be utilised practically as premonitory indicators of the "nutritional" break-down of stock animals.

Apart altogether from the data elicited from this work which bears on the study of nutrition, much of the information obtained is of interest from a general biological standpoint and contributes to our knowledge of natural serum reactions.

At the commencement of this research certain serum reactions were selected for testing purposes in virtue of their known occurrence in measurable degree with sheep serum. The tests were, of course, quantitative. An attempt was made to restrict the tests to organisms which were pathogenic to sheep, but these were found to give very feeble reactions and the limits had to be extended.

The/

The reactions studied were as follows:-

(1) The bactericidal effects of serum towards selected strains of B. coli and a strain of B. suipestifer, which depend on natural antibodies acting along with serum complement.

(2) Bactericidal effect of serum towards a strain of Streptococcus haemolyticus, which depends on the so-called β -lysin acting independently of complement.

(3) Haemolytic action of serum towards rabbit erythrocytes, due to a natural haemolytic antibody and serum complement. (Mackie & Finkelstein 1931).

(4) Complementing action of serum for a haemolytic system of ox erythrocytes and a goat versus ox immune antibody.

(5) Agglutinating effect of serum towards selected strains of B.abortus and B. paratyphosus B, due to natural agglutinating antibodies acting independently of complement.

It should be emphasised that no attempt was made to differentiate between the bactericidal or haemolytic antibody and complement in reactions (1) and (3). Owing to technical difficulties it was not found possible to carry out the titration of complement until the last few samplings. Thus when "haemolysin" is referred to throughout the thesis, it implies the natural haemolytic action of the serum due to both antibody and complement.

Some of these reactions have been used more than others.

Many difficulties have presented themselves in the course of this work. Individual animals vary in the strength of their reactions, and the variation is more pronounced with some reactions than others. Reactions vary according to the season of the year (as has been shown by others (Blake & Okell, 1929,

Kraus & Chairmont, 1900, Tromsdorff, 1902 and Mackie & Finkelstein 1932)), and under certain conditions they appear to vary from day to day. The serum principles are in some cases labile and tests have necessarily been carried out within the minimum time after bleeding (usually sixteen hours). Further, the biological reagents used, e.g. erythrocytes, bacterial cultures, are subject to variation.

Nutritional Experiments. Sheep were the most suitable animals for these experiments on account of their uniformity, the ease with which they can be handled and because they show much greater differences in weight under dietary influences and supply a larger quantity of blood than other uniform animals (e.g. white rats).

Of the first two nutritional experiments in which the writer has participated, one was carried out at Ashtown, one of the Institute's farms at Aberdeen, and the other at Garrochoran, Argyllshire.

The farm of Garrochoran was acquired by the Rowett Institute as a typical example of a Scottish hill farm on which the pasture is of poor quality and where the animals are characterised by stunted growth, high mortality and low rate of reproduction. The poor pasture is doubtless due to impoverishment of the soil which, in turn, has been brought about by the sheep-raising methods carried out for many years by past generations of farmers. Briefly, these were to bring up as many sheep as possible on the pasture and to return nothing to it. The inevitable result, which is being felt at the present time, is the diminishing return from the pasture in the form of carcasses of sheep. The withdrawal of these carcasses from the farm means permanent loss from the soil of those elements such as Ca, P, Na which the animals have taken from the pasture and from the soil and which cannot be recovered from the surrounding atmosphere. The use of appropriate manures and fertilizers serves to repair this loss, but in districts where this is economically impossible a serious problem arises. On such farms it has been noticed that the sheep show a preference for certain parts of the pasture and the following table (Godden 1926) shows/

shows the average composition of such pastures as compared with cultivated pastures.

Table I.

Average Composition of Scottish Hill Pastures.
Results expressed as Grams per cent of Dry Matter.

	"Grass Eaten"	"Grass not Eaten"	"Cultivated Pasture"
CaO	0.56	0.30	1.00
P_2O_5	0.60	0.37	0.74
Na_2O	0.41	0.17	0.25
K_2O	2.60	1.61	3.18
Cl	0.60	0.33	0.95
N	2.54	1.82	2.83
Silica = free ash.	5.49	3.13	6.64
Fibre	25.2	29.30	23.00

These figures for "grass eaten" and "grass not eaten" were obtained chiefly from Argyllshire and neighbouring counties in the West of Scotland, where the sheep are small, the mortality rate is above 10 per cent, and the rate of reproduction low. (Reid 1916). Such, then, were the conditions prevalent at Garrochoran, where overstocking occurred with 500 sheep on 1200 acres (a good pasturage will carry three sheep per acre).

The poorly nourished sheep on this farm were thus excellent material for testing the influence of nutrition on immunity of animals under natural conditions. The farm was, however, at some considerable distance from the laboratory and difficulties as to transport of samples, etc. arose. It was, therefore, decided to carry out an experiment nearer the laboratory where conditions could be more closely controlled.

The general scheme of the experiments was as follows:-

Ashtown. Several groups of sheep, comparable as regards age, sex, and previous history, were kept under artificially confined conditions. These were fed on

on diets prepared in such a way as to be deficient in one or more of the constituents in which the pasture of poor farms is lacking.

Garrochoran. In this experiment large numbers of sheep, living under the usual conditions of a hill farm, were kept under observation. These were divided into groups and supplementary feeding was given in an attempt to make up the deficiencies known to be present in the pasture.

The present thesis is divided into several sections, of which section I deals with the details of immunological technique. Sections II and IV are devoted to a general account of the observations made in the Ashtown and Garrochoran experiments respectively. In these two sections the results are stated in a descriptive fashion, as they appeared before a critical statistical analysis had been made. It is, however, obvious that,to obtain any idea of the importance to be attached to any particular observation and to find out what apparent effects are due to mere chance variations, it is necessary to undertake a statistical analysis of the figures which have been obtained. Some account of this analysis is presented in sections III and V, dealing respectively with the Ashtown and Garrochoran experiments. In section VI some account is given of intradermal toxin tests carried out in the experimental animals and of the statistical analysis of the figures so obtained. This is followed in section VII by a general discussion of the results and of the conclusions to be drawn therefrom.

With/

With regard to the statistical analysis of the results, especially those dealt with in section III, a few general observations may appropriately be made here. The serological findings, as a consequence of their nature and the techniques employed, are only very approximate as far as the numerical values which have been obtained are concerned. Furthermore, the number of animals employed in each particular group is, in practically every case, fairly small. In addition to and partly possibly in consequence of these two circumstances many of the apparent differences are on inspection readily seen to be without statistical significance and due simply to random variation. It follows that the data are of such a nature as to make the application of extremely refined and exhaustive statistical methods of analysis inappropriate and unlikely to yield any significant results which would not be given by rougher and less time-consuming methods. In view of the large amount of data to be examined, it has been necessary to rely, in the first instance, on relatively simple tests and where it seemed desirable, to follow up any indications which these might afford by rather more complicated methods. Though this treatment may leave something to be desired from the point of view of a rigorous and exhaustive statistical analysis, it is, nevertheless, probable that most of the significant features have been detected and it seems dangerous to press too far crude data of the type under discussion.

SECTION I.

IMMUNOLOGICAL TECHNIQUE.

(1) Bactericidal Reactions with B. coli and B. suipestifer. (Method I of Mackie & Finkelstein. (1931)).

In these tests a standard emulsion of the test organism was prepared and from this was made a series of dilutions in 0.85 per cent saline. A given volume of serum was then mixed with a given volume of each dilution in small stoppered sterile test tubes, a control series being included in which the serum was replaced by an equal volume of sterile saline. The tubes were incubated for the requisite time and on removal from the incubator a standard loopful was removed from each and a stroke inoculation made on the appropriate medium. The amount of growth resulting from each inoculation was noted after 24 and 48 hours incubation, and by comparing the end-point in the serum series with that in the saline controls, an indication of the relative sterilizing effect of the different sera could be obtained.

A control of the relative sterility of each serum was also included. In this tube the bacterial emulsion was replaced by an equal volume of sterile saline.

Strictly aseptic precautions were necessary throughout these tests.

Reading of the bactericidal reactions. The approximate amount of growth in each tube was noted, empirical standards being employed:-

4 = confluent growth.
3 = discrete colonies but heavy growth.
2 = discrete colonies less growth than 3.
1 = discrete colonies slight growth.
fc= few colonies (6 - about 12)
With less than 6 colonies the actual number was noted.

Comparison was then made of the position of the end-point of growth in each serum series with that in the saline control series and the logarithm of the ratio of the latter to the former i.e. the difference between the indices of the dilutions, was taken as the index of bactericidal power of the serum, and will be hereafter referred to as indicating so many "units of bactericidal action" or "bactericidal units". The readings from typical series are shown below:-

	* S/1	S/10	$S/10^2$	$S/10^4$	$S/10^6$	$S/10^8$	
Saline	4	4	2	1	fc	0	
Serum I	4	2	1	0			bactericidal power = 4 units
Serum II	4	2	1	5c	0		" " = 2 "
Serum III	4	4	2	1	3c	0	" " = 0 "
Saline	4	2	1	0			
Serum	3	1	0	0			" " = 1 "

(* S = Standard Emulsion)

Bactericidal reaction with B. suipestifer. The standard emulsion (S) of this organism (prepared from 24 hour agar slope cultures) was equal to Brown's opacity tube 2, and the series of dilutions was: S. S/10. $S/10^2$. $S/10^4$. $S/10^6$. $S/10^8$. The test amount of serum was 0.15 c.c. and of bacterial emulsion 0.5 c.c. Incubation was allowed to proceed for three hours and the transfers were made on to McConkey's medium. The plates were divided into twelve, so that two series of transfers might be made on each plate.

Bactericidal reaction with B. coli "X". 24-hour agar slope cultures were used for the preparation of the emulsion, which in this case was equal in opacity to Brown's tube 1. The series of dilutions was S. $S/10^2$, $S/10^4$. $S/10^6$. $S/10^8$. $S/10^{10}$., but later the first tube was omitted, as the range of/

of bacteriolytic action fell within the limits $S/10^2$ and $S/10^{10}$. The test amounts were again 0.15 c.c. and 0.5 c.c. of serum and emulsion respectively, the time of incubation 3 hours, and McConkey's medium was used for plating.

After the above technique had been used for some time, it was found that consistently negative results were being obtained. The quantities of serum and bacterial emulsion were therefore altered to 0.25 c.c. of each, the dilutions of the latter used being $S/10^2$, $S/10^4$, $S/10^5$, $S/10^6$ and $S/10^7$. The time of incubation was increased to 24 hours. This necessitated dispensing with the saline control series and plating from all tubes before and after incubation. This alteration in technique was made in November, 1931.

(2) Bactericidal reaction with Streptococcus haemolyticus.

This reaction is due to β -lysin acting independently of complement. As the viability of this organism decreases in saline, a different technique was required from that for the tests with B. coli and B. suipestifer. The saline control tubes were not included and stroke inoculations were made before and after incubation.

The organism was grown in phosphate bouillon for 24 hours, the supernatant bouillon poured off, and the sedimented growth emulsified in sufficient broth to make a standard equal to Brown's opacity tube No.5. From this emulsion dilutions were made in 0.85 per cent saline to give the series S, $S/10^2$, $S/10^4$, $S/10^6$, $S/10^8$. In November, 1931 this series was modified to S, S/10, $S/10^2$, $S/10^4$, $S/10^6$, $S/10^8$.

0.5 c.c. was the test amount of serum, and to this was added 0.15 c.c. of bacterial emulsion. Loop transfers were made on to blood digest agar before/

before and after incubating for four hours. In November, 1931 the medium was changed to blood agar, and the time of incubation to 24 hours. The change of medium was made for convenience in working while the period of incubation was altered in an attempt to get positive bactericidal effects.

(3) Haemolytic reaction with Rabbit Erythrocytes.

This reaction is due to the action of natural haemolysin and complement in the serum on a suspension of washed rabbit erythrocytes.

The cell suspension was prepared by collecting blood from a rabbit's ear and defibrinating with glass beads. The cells and plasma were poured off into a volume of 0.85 per cent saline roughly equal to that of the blood, and centrifuged. The supernatant fluid was discarded and the cells washed twice with saline, sufficient to make up to the original volume. The period of centrifuging was seven minutes each time, at about 3000 revolutions per minute. A 3 per cent suspension of the deposited cells, after the last centrifuging, was made in 0.85 per cent saline.

Varying amounts of serum were placed in small test tubes and sufficient saline added to make up the volume to 0.5 c.c. To each of these was added 0.5 c.c. cell suspension and the tubes were then incubated at 37°C for one hour. They were shaken after 40 minutes to stir up the cells, as these tend to deposit at the foot of the tube. Readings of the amount of lysis which had occurred were made on taking the tests out of the incubator, and again the following morning. The readings were recorded as "trace","distinct","marked", "very marked", "almost complete", or "complete". The amount of serum necessary to produce complete lysis was taken as the Minimum Haemolytic Dose (M.H.D.).

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In the case of a serum giving a reading of "almost complete" in one tube, the M.H.D. was taken as the amount intermediate between that in the tube giving this reading and in the tube showing complete lysis, e.g.:-

Volume of serum		0.1	0.2	0.3	0.4 c.c.
Lysis		m	vm	c	c
M.H.D.	=	0.3 c.c.			

Volume of serum		0.1	0.2	0.3	0.4 c.c.
Lysis		m	ac	c	c
M.H.D.	=	0.25 c.c.			

Some sheep gave a much lower haemolytic titre than others and then it was inconvenient to work with 0.5 c.c. of cell suspension, therefore, only 0.25 c.c. was used. In these cases, in order to keep records uniform, the M.H.D. recorded was that required for 0.5 c.c. of erythrocytes, i.e. twice that actually observed.

(4) Complementary activity of sheep serum.

Owing to the difficulty of finding a satisfactory method of absorbing normal haemolysin from sheep serum, a system had to be selected for which sheep serum had no haemolytic activity. The most convenient system was ox erythrocytes, and it was necessary to prepare a goat v. ox haemolytic immune body.

A three per cent suspension of ox erythrocytes was prepared in the same way as the rabbit erythrocyte suspension for test 3 above. This suspension was sensitized by adding an amount of immune body equal to five times the M.H.D. for the volume of erythrocytes to be prepared. 0.25 c.c. of this sensitized suspension was used as the test quantity for finding the M.H.D. of complement in the sheep serum.

The reading of the tests was similar to that in test 3, the M.H.D. recorded/

recorded again being that for 0.5 c.c. suspension.

(5) Agglutination Tests.

The usual technique was employed for these tests. Dilutions of the sera with normal saline in geometrical progression ($\frac{1}{X}$, $\frac{1}{2X}$, $\frac{1}{4X}$, ...), were made, 0.2 c.c. being the final quantity of each dilution. To each of these dilutions was added an equal volume of emulsion of the test organism making the dilution series ($\frac{1}{2X}$, $\frac{1}{4X}$, $\frac{1}{8X}$, ...). The mixtures were transferred from the 3" x $\frac{1}{2}$" tubes in which the dilutions were made, to 3" x $\frac{1}{4}$" tubes and incubated for 24 hours. On removal from the incubator, the tubes were allowed to stand for a few minutes before readings were made. The amount of agglutination occurring was noted in each tube as below, and the highest dilution in which any agglutination occurred was taken as the end-titre of the serum.

4 = agglutination with complete clearing of supernatant fluid.

3 = agglutination, but supernatant fluid not quite clear.

2 = marked agglutination.

1 = trace of agglutination.

Emulsions of live organisms were originally used for the agglutination tests, but these were replaced by formalised suspensions at the second sampling. At the third and subsequent samplings, however, live organisms again were used. The change to formalised suspensions was made in order to ensure stricter uniformity, but as the natural agglutinogens are said to be destroyed by formalin, their use was abandoned.

Difficulty was experienced in finding a suitable index for the agglutinating power of a serum, which could be used in working out averages and in graphical representations. If the direct /

direct dilutions are used, e.g. 1/8, 1/16, 1/32, ...1/256, a skew curve results. This is remedied if those figures are replaced by others denoting the power to which 1/2 must be raised to give the required number, i.e. a dilution of 1/8 is represented by 3, 1/32 by 5, and so on. The resulting curve is much more normal than that obtained when the dilutions themselves are used. If was therefore considered that these figures might be used as an index of agglutinating power, and they are hereafter referred to as units of agglutination.

Agglutination Test with B. paratyphosus B. Growth on agar plates incubated for 24 hours was used to prepare the emulsion of this organism, which was standardised to Brown's opacity tube No.4. The original series of serum dilutions was ($\frac{1}{2}$, $\frac{1}{4}$.....$\frac{1}{64}$), and after the addition of the bacterial emulsion ($\frac{1}{4}$, $\frac{1}{8}$,.....$\frac{1}{128}$).

Agglutination Test with B. abortus (Hog). In order to obtain sufficient growth of this organism, agar plates were incubated for 48 hours. The opacity of the emulsion was that of Brown's tube No.4. In the first few tests the original serum dilutions were ($\frac{1}{2}$, $\frac{1}{4}$,.....$\frac{1}{64}$), but these were later changed to ($\frac{1}{4}$, $\frac{1}{8}$,.....$\frac{1}{128}$).

Sampling of the Blood and Preparation for Tests. The actual sampling of the blood was done by means of a needle (Samco No. $^{1}/_{1}$-3"long) inserted into the jugular vein of the animal. After rejecting the first few drops of blood the requisite amount was collected in stoppered, sterile centrifuge tubes (25 c.c. capacity).

The needles were thoroughly cleaned with soap and water and then with olive/

olive oil and ether and were placed in containers with liquid paraffin. The containers were sterilised by steaming for 1½ hours. When required for use, the needle was withdrawn with sterile forceps, heated gently in the flame to liquify the paraffin, which was then shaken out as far as possible. The coating of paraffin left in the needle allowed the large quantity of blood required, 50 - 60 c.c., for those and biochemical tests to flow through it without clotting.

When several hours would elapse before their arrival at the laboratory, the tubes of blood were packed in an ice-box for transport. On receipt in the laboratory the clot was separated from the side of the tube and centrifuged. Strictly aseptic precautions were observed until the bactericidal tests were set up, this being done immediately.

The period elapsing between the commencement of sampling and the arrival of the blood in the laboratory varied from 3¼ hours when the blood was sampled at Aberdeen, to 12 hours when sampled in the West of Scotland and transported to Edinburgh or Aberdeen. Preliminary trials were carried out to determine whether the results were affected by the length of time the drawn blood was kept before testing it, and though this did not seem to be the case when the time was short, the tests were in every case carried out as soon as the transport of the samples permitted. This varied according to whether the animals were at Aberdeen or some other part of the country, but the lytic tests have always been completed within 24 hours of the commencement of drawing blood except on two occasions. The agglutination tests were carried out the following morning.

SECTION II.

ASHTOWN EXPERIMENT.

Eighty half-bred (Border Leicester-Cheviot first cross) wether hoggs * about seven months old at the commencement of the experiment in November were selected. These had been bred at the Institute, and since the time of weaning in August had been grazing on the same pasture. Thus their previous nutritional history was identical, except for unavoidable differences which might have been present in the mother's milk.

After a preliminary bleeding, to obtain normal blood values for the reactions, the sheep were separated into sixteen groups of five, each group being comparable as regards age, size, weight and quality. Eight groups were housed in special buildings at Ashtown Farm, while the other eight were kept in netted enclosures free from grass, or later, when the young grass appeared, in gravelled sheep pens. The indoor animals were provided with moss litter and, as one end of the building received more ventilation and light than the other, each group was moved daily into the pen occupied by the preceding group on the previous day. This ensured that the housing conditions were similar for all groups.

The basal ration was considered to be low in calcium and nitrogen, as it contained less of these elements than the "grass eaten" of hill pasture. The relative amounts of these elements in the basal ration and a corresponding ration/

* (Castrated males between 6 and 18 months old)

ration of hill pasture are shown in the following table, the figures being calculated on a dry matter basis.

Table 2.

	N.	Ca.	P.	Cl.
3 lbs. Basal Ration) contains in grams.)	22.9	2.36	5.94	3.0
3 lbs. Natural Pasture) contains in grams.)	33.7	5.79	3.54	8.1

The basal ration was thus deficient in nitrogen, calcium, and chlorine, while it contained a relative excess of phosphorus. The chlorine deficiency was made good by the provision of salt-licks for all groups.

The basal ration was supplemented with:

(a) calcium (as chalk).
(b) protein (as cottonseed meal).
(c) cod liver oil,

separately or in combination. The following table shows the rations of each group:

Table 3.

Group 1.

Basal Diet:	
Straw	ad lib.
Turnips	do:
Maize	225 parts.
Bran	225 "
Dried grains	225 "
Oats	225 "

2	3	4	5	6	7	8
Basal diet + 10 c.c. C.L.O.	Basal diet + 20 pts. Chalk	Basal diet + 20 pts. Chalk + 10 c.c. C.L.O.	Basal diet + 225 pts. C.S.M.	Basal diet + 225 pts. C.S.M. + 10 c.c. C.L.O.	Basal diet + 20 pts. Chalk + 225 pts. C.S.M.	Basal diet + 20 pts. Chalk. + 225 pts. C.S.M. + 10 c.c. C.L.O.

C.L.O. = Cod Liver Oil
C.S.M. = Cottonseed meal.
Salt licks to all groups ad lib.

The ration for each sheep was 0.5 lbs. of cereals per day, increased in a few days to 1 lb. per day, as the previous quantity proved inadequate. The turnips, which were cut, were finally apportioned at 8 lbs. per head per day for inside sheep, and 12 lbs. for outdoor animals.

Early in the experiment it was noticed that the sheep in groups 5 and 6 (i.e. calcium deficient groups) ate their ration of straw much more eagerly and voraciously than the others; their rations of this material, therefore, had to be reduced in case the calcium deficiencies of the ration might be compensated for by the calcium present in the straw. By the end of June the turnip supply became exhausted and potatoes were substituted. This rendered the diet more unbalanced in regard to the calcium and phosphorus.

By June several of the sheep were in such bad condition that it was considered inadvisable to keep them on experiment longer and they were turned out to grass. They were bled in September, together with those still on experiment.

Originally 8 sheep were bled each day, one from each of either indoor or outdoor groups, but later as the workers became more efficient, the number was increased to 16 per day (2 from each group). Five samplings were required to complete the examination of all the animals and these were usually carried out on Mondays and Thursdays. Indoor and outdoor groups were taken alternately, till the last occasion when representatives were taken from both outdoor and indoor groups. The actual dates of sampling were as follows:-

November/

November. 18,20,22,25,28,29. December 3.
January. 14,16,20,22,24,27,30. February 3,7.
March. 24,27,31. April 3,7.
May. 19,22. June 12,16,19. (repeats on 26th)
September. 10,15,17,23.

The amount of blood taken from each sheep was usually between fifty and sixty c.c. 25 c.c. of this being required for immunological tests, the remainder for biochemical and haemocytological determinations which were carried out concurrently. The sampling of the blood was usually commenced at 10 a.m. Depending on the time taken to bleed, the centrifuging of the tubes began at 2-3 p.m.

RESULTS.

The complete results are tabulated in the Appendix.

In December, when the pre-experimental examination was made, the technique was not sufficiently mastered and there are many blanks in the results. At this time only three tests were being carried out, viz: haemolytic reaction with rabbit erythrocytes, bactericidal reaction with B. suipestifer, and agglutination reaction with B. paratyphosus B, though ten animals at the end of this period were also tested for their agglutinating power to B.abortus(Hog).

The results of these tests were more uniform than had been expected, the variations being as below.

Haemolytic reaction, M.H.D. 0.2-0.35 c.c. i.e. No. of M.H.D.s per c.c. 5.0-2.8

Bactericidal reaction with B.suipestifer 10 to 6 bactericidal units *

Agglutination reaction with B. paratyphosus B, 1/4 to 1/64 (only 2 readings of 1/4 and one of 1/64).

The distribution was as follows:-

Table 4.

Haemolysin						No. of animals tested.
M.H.D.s per c.c.	5	4	3.3	2.8		
Percentage	30.4	25	30.4	13.8		36
Bactericidal reaction with B.suipestifer.	6	7	8	9	10	
Percentage	18	0	33	22.5	25.5	66
Agglutination reaction with B.paratyphosus B.	1/4	1/8	1/16	1/32	1/64	
Percentage.	3.5	19.25	45.5	29.75	1.75	57

In January two additional tests - bactericidal reaction with B.coli "X" and agglutination test with B.abortus (Hog) - were added to those above. The

* At this time the end-point in this reaction was not satisfactory and these results are not comparable with later ones.

The ten animals tested with B. abortus in December gave end-points 1/64 and 1/256.

The agglutination reactions in January differed from those of any other period, as at this time formalised suspensions of the organisms were used, as explained on page 16.

Many factors other than purely dietary ones must be taken into consideration in examining the results. Individual, daily, and seasonal variations complicate these while ultra-violet light and environment (outdoor or indoor) may have influenced them.

During the winter months the indoor animals suffered from foot-rot.

Bactericidal Reaction with B. suipestifer.

Indoor Groups. Table V. shows the average figures for the groups at the various dates of sampling. Little difference appeared between the groups at any time. The controls for this test during the December samplings were not satisfactory and, therefore, there was more variation at that date than at any other. When the animals kept on experiment and those turned out to grass are compared in September a very marked difference is seen, the results in the case of the former animals having fallen to a lower level than previously while, in the case of the sheep on grass, they have risen higher than the January figures.

It is remarkable how closely the figures for groups I and VIII approximate throughout the experiment until May. Unfortunately, they cannot be compared in September as it had been found necessary to put all the sheep in group I to grass.

The/

The general level of the results varied from month to month. No appreci-:able difference occurred between January and March but a slight fall is noticeable from March through May to September.

Outdoor Groups. As with the indoor animals no group differences are noticeable until September. In that month, though the differences are not striking, the groups receiving protein (i.e. V - VIII) gave higher results than those receiving no protein, except group IV which was highest of all. This difference, however, is probably due to sampling on different days. Again the sheep on grass in September are higher than those on experiment.

The drop from month to month is hardly so marked in these animals as in the indoor ones: indeed the groups receiving protein (V - VIII) remain remarkably steady, and group IV, though it falls from January through March to May, rises again in September. Groups I-III show a progressive fall.

Comparing the levels of the reaction in indoor and outdoor animals from month to month, it is seen that in January the outdoor results are slightly higher than the indoor ones. In March this difference is not obvious, but in May and September it again appears.

Bacteriolysis of B. coli "X".
Table VI.

No pre-experimental figures are available for this reaction, as it was not included among the tests until the second bleeding in January.

Indoor Groups. Group IV. was the highest in January, with groups II and VIII a little lower. In March again it was highest, while groups III, V and VIII/

Fig 1.

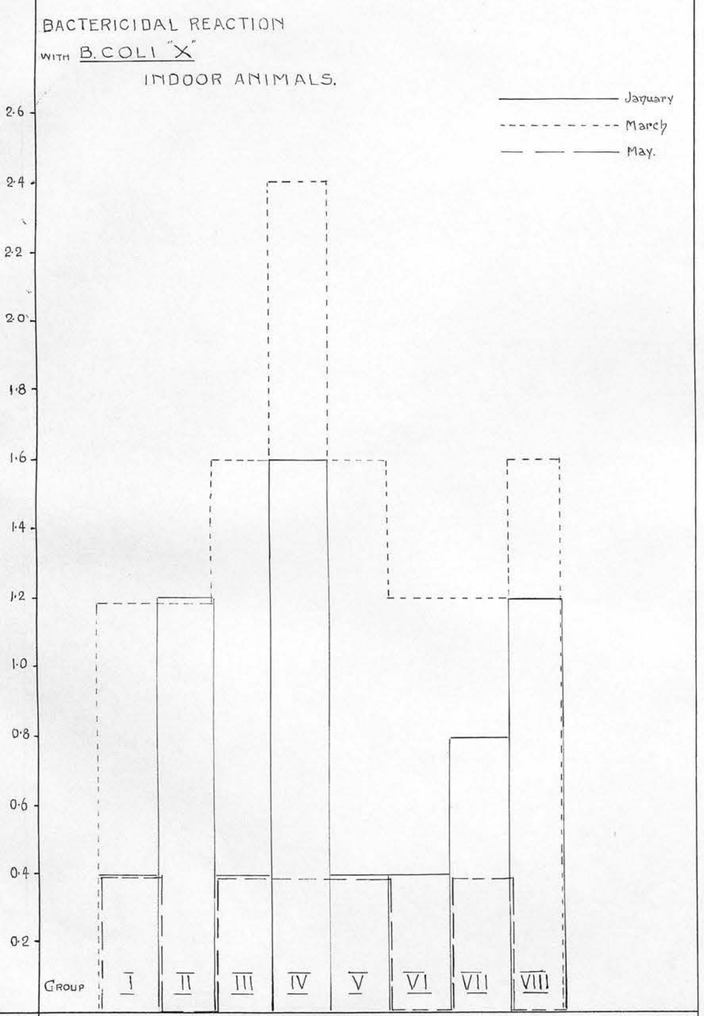


Fig 2.

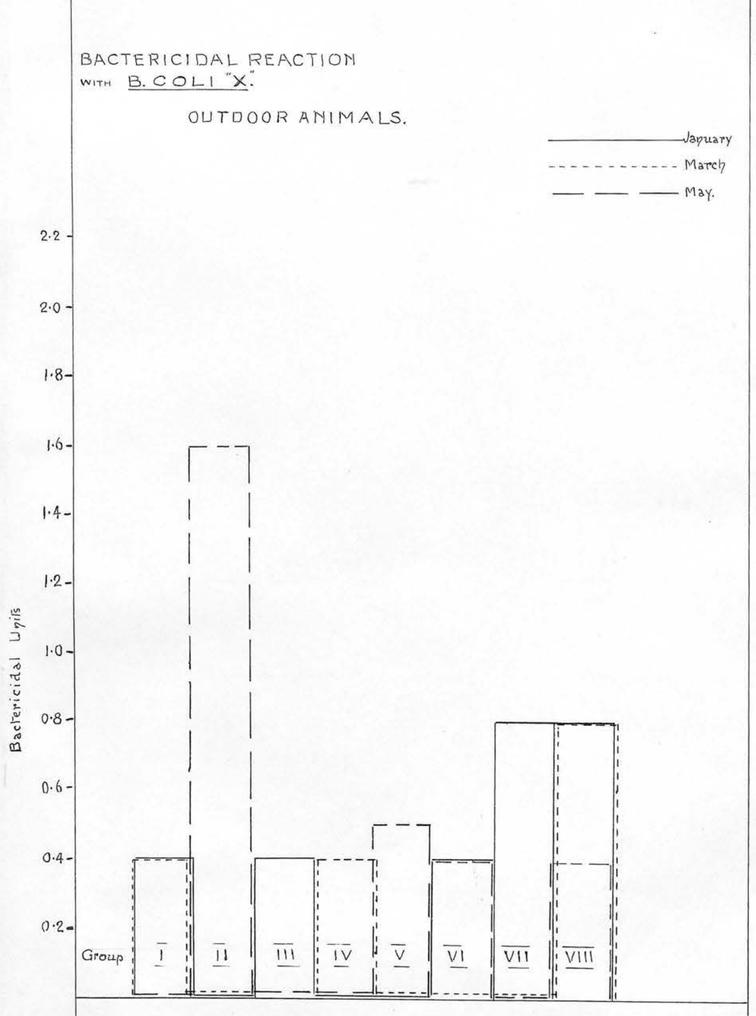
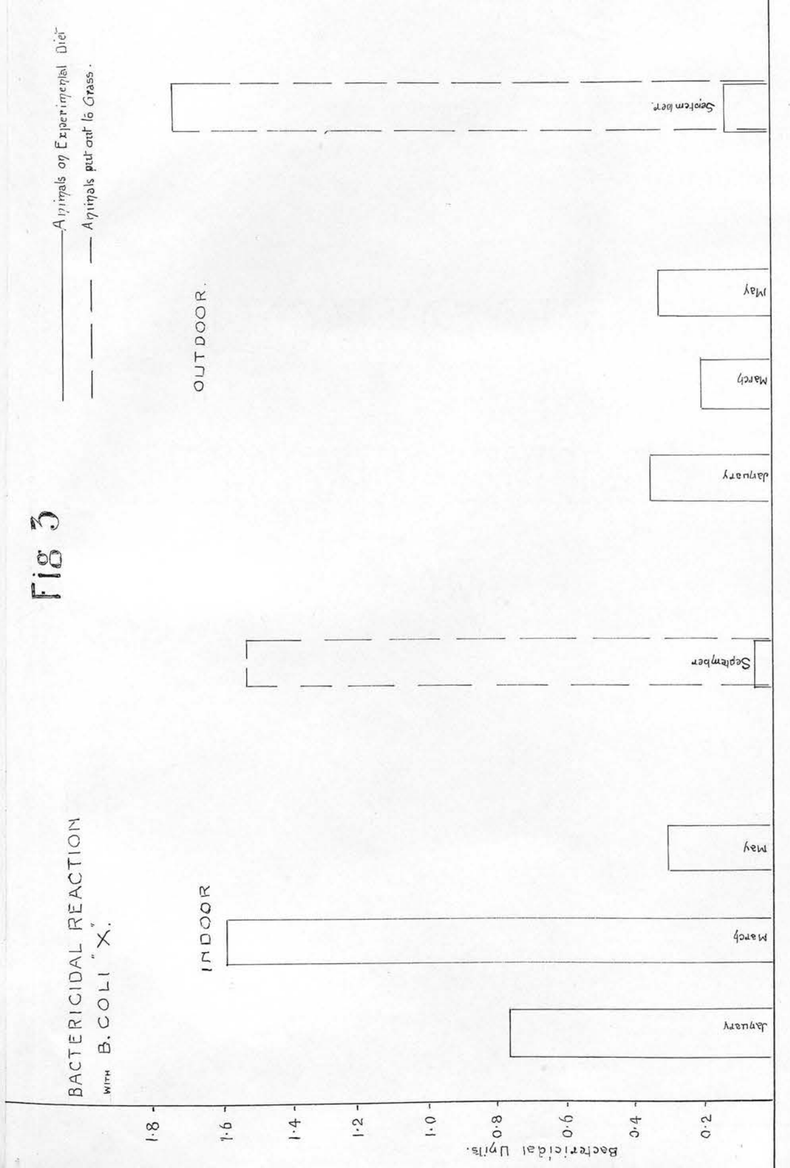


Fig 3



VIII were slightly higher than the remaining four groups (fig.1). By May all the figures had fallen considerably and there was no real differentiation between the groups. In September, except for one sheep, all those still on experiment gave negative results while those transferred to grass gave positive figures.

The general level of the reaction in March had risen somewhat from the January figures, and this was followed by a considerable fall from March to May.

Outdoor Groups. Groups VII and VIII were highest in January and group VIII followed by I and IV in March. In May group II was highest, with V, then VI and VIII, next in order (fig.2). By September all but two animals on experiment showed no bactericidal action to B.coli "X" while five of the six on grass gave positive results.

The level from month to month was much steadier in the outdoor than the indoor animals only a very small drop occurring as the experiment progressed.

When the reaction levels in the outdoor and indoor groups are compared a marked difference is noticeable (fig.3). In January the average of all indoor animals was 0.75 as compared with an outdoor average of 0.35, i.e. a ratio of 2.1 : 1. In March these averages had changed to 1.5 and 0.2 respectively, the ratio becoming 7.5 : 1. By May they were 0.25 and 0.32, the differences being wiped out. It is not possible to tell what occasioned this great difference.

Haemolytic Reaction with Rabbit Erythrocytes.
Table VII.

In/

TABLE V.

ASHTOWN EXPERIMENT.

BACTERICIDAL REACTION WITH B. SUIPESTIFER.

GROUP AVERAGES.

(in bactericidal units)

Indoor Groups

Group I.	1929. Dec.	1930. Jan.	Mar.	May.	September. Animals Diets	September. on Grass.
I.	7.4	4.4	3.8	3.5(4)	-	4.5(4)
II.	8.5(4)	4.2	4.4	2.7(3)	2.7(3)	6.5(2)
III.	8.2	4.2	4.2	4.0(3)	1.0(2)	4.7(3)
IV.	9.2(4)	4.2	4.4	2.7(3)	2.5(4)	5.0(1)
V.	9.3(3)	4.4	4.2	2.7(3)	2.3(3)	-
VI.	9.5(4)	4.2	4.0	2.7(3)	2.5(4)	5.0(1)
VII.	8.0(3)	4.0	4.2	3.3(3)	3.5(2)	4.6(3)
VIII.	7.5(4)	4.4	3.6	3.3(3)	3.0(3)	5.0(1)
All groups	8.4	4.25	4.1	3.1	2.5	5.0

Outdoor Groups

Group.						
I.	8.0(4)	4.4	4.1	4.0(3)	2.7(4)	5.0(1)
II.	8.8	4.0	4.2	3.3(3)	2.7(4)	-
III.	8.8	4.6	4.2	3.3(3)	3.0(4)	5.0(1)
IV.	9.7(4)	4.6	3.8	3.0(2)	4.5(4)	5.0(1)
V.	8.4	4.6	4.2(4)	4.0(2)	4.0(4)	-
VI.	6.0(3)	4.8	4.4	4.0(3)	4.2(4)	5.0(1)
VII.	8.0(4)	4.6	4.2	3.3(3)	4.0(2)	5.0(1)
VIII.	8.5(4)	4.6	4.4	4.0(3)	4.2(4)	5.0(1)
All groups	8.4	4.5	4.2	3.6	3.6	5.0

(The average for each group is compiled from 5 results except where indicated by a figure in brackets).

Fig 4.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES.

INDOOR GROUPS.

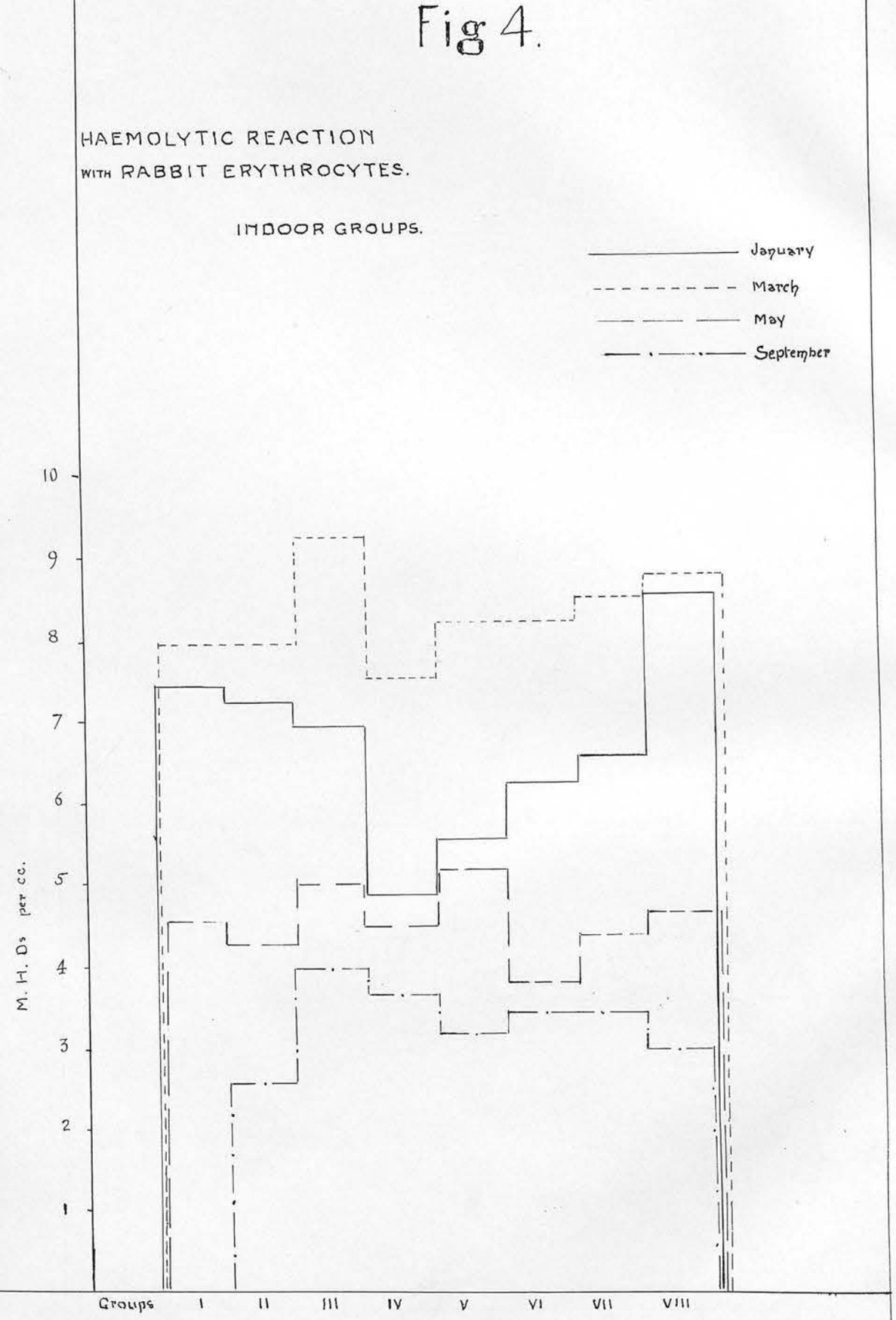
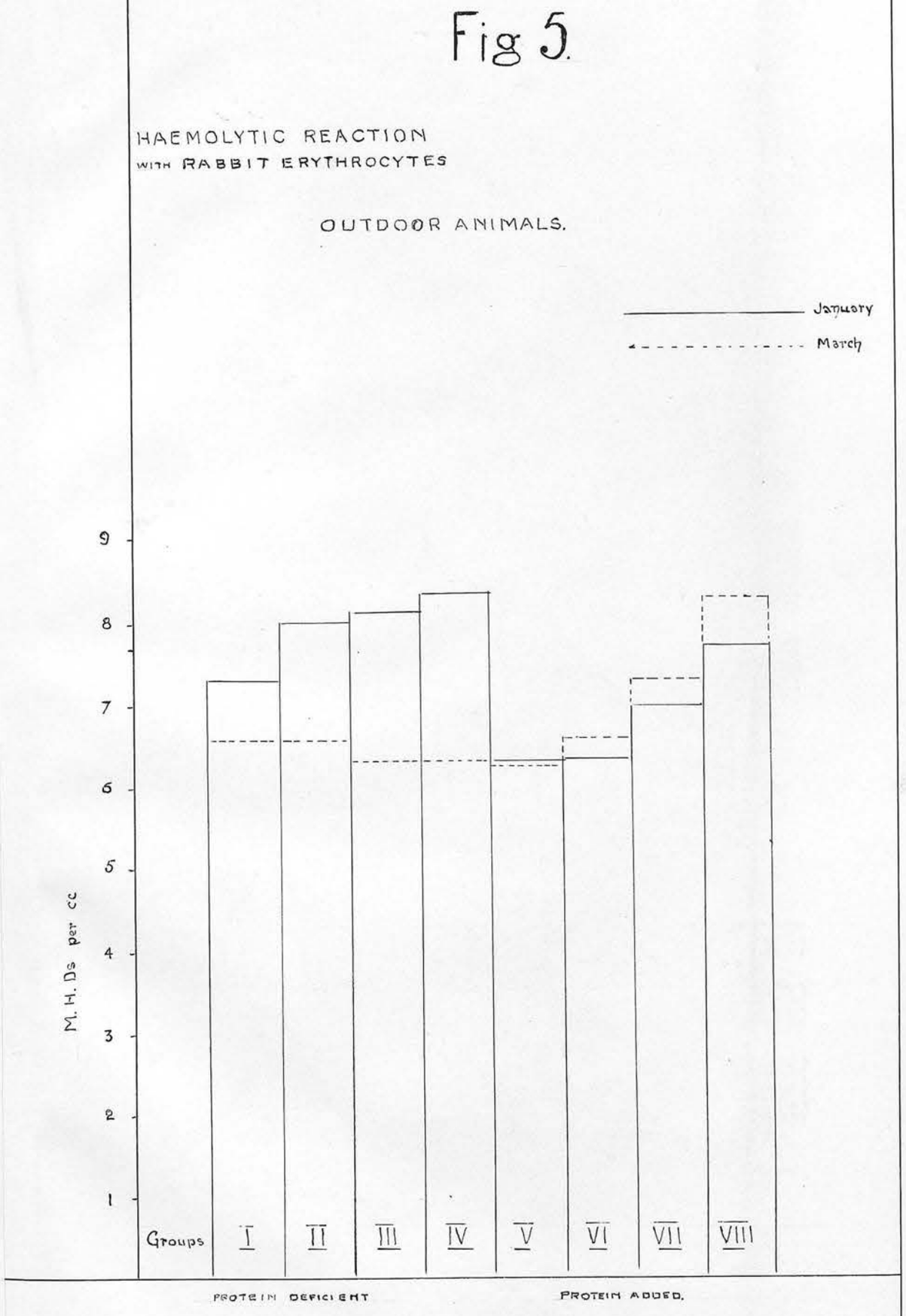


Fig 5.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES

OUTDOOR ANIMALS.



In December when the pre-experimental readings were taken, there was remarkably little variation between the groups. Unfortunately, all animals were not tested at this time, and there were no representatives of groups V indoors and VI outdoors among those tested.

Indoor Groups. Groups II, VII, and VIII gave highest figures for this reaction in December. By January group VIII was highest if an aberrant value in group IV be disregarded. Groups I, II and III were next in order, while group IV was lowest. The highest figure in March was obtained in group III, while the lowest was again in group IV. There was little difference between the groups at this time, and indeed at the remaining samplings. In May group V was highest, with III slightly lower and group VI lowest, and in September group III was highest and II was lowest (fig.4). The average of the animals put on to grass was higher than that of the experimental animals in September, being 3.3 and 4.1 M.H.D.s respectively.

The figures for all groups show a rise from December through January to March and then a fall from March to May and September.

Outdoor Groups. Group VIII gave the highest average when the pre-experimental tests were made, while group II gave the lowest figure. In January group IV was highest and groups V and VI lowest. It may be remarked that at this time the order of the groups was VI, V, VII, I, VIII, II, III, IV, i.e. the groups receiving protein gave lower figures than those deficient in this substance, with an overlap between the highest group of the one series and the lowest group of the other. The order of the groups receiving supplements is the same in both series (fig.5). By March this difference had quite disappeared, very little/

In December when the pre-experimental readings were taken, there was remarkably little variation between the groups. Unfortunately, all animals were not tested at this time, and there were no representatives of groups V indoors and VI outdoors among those tested.

Indoor Groups. Groups II, VII, and VIII gave highest figures for this reaction in December. By January group VIII was highest if an aberrant value in group IV be disregarded. Groups I, II and III were next in order, while group IV was lowest. The highest figure in March was obtained in group III, while the lowest was again in group IV. There was little difference between the groups at this time, and indeed at the remaining samplings. In May group V was highest, with III slightly lower and group VI lowest, and in September group III was highest and II was lowest (fig.4). The average of the animals put on to grass was higher than that of the experimental animals in September, being 3.3 and 4.1 M.H.D.s respectively.

The figures for all groups show a rise from December through January to March and then a fall from March to May and September.

Outdoor Groups. Group VIII gave the highest average when the pre-experimental tests were made, while group II gave the lowest figure. In January group IV was highest and groups V and VI lowest. It may be remarked that at this time the order of the groups was VI, V, VII, I, VIII, II, III, IV, i.e. the groups receiving protein gave lower figures than those deficient in this substance, with an overlap between the highest group of the one series and the lowest group of the other. The order of the groups receiving supplements is the same in both series (fig.5). By March this difference had quite disappeared, very little/

TABLE VI.

ASHTOWN EXPERIMENT.

BACTERICIDAL REACTION WITH B. COLI "X"

GROUP AVERAGES.

(in bactericidal units)

Indoor Groups.

Group.	1930. Jan.	Mar.	May.	September Animals on Diets.	September Animals on Grass.
I.	0.4	1.2	0.4	-	2.0(4)
II.	1.2	1.2	0.0	0.0(3)	1.0(2)
III.	0.4	1.6	0.4	0.0(2)	1.3(3)
IV.	1.6	2.4	0.4	0.0(4)	2.0(1)
V.	0.4	1.6	0.4	0.0(3)	-
VI.	0.0	1.2	0.0	0.0(4)	2.0(1)
VII.	0.8	1.2	0.4	1.0(2)	1.3(3)
VIII.	1.2	1.6	0.0	0.0(3)	0.0(1)
All groups.	.75	1.5	.25	.048	1.45

Outdoor Groups.

Group.					
I.	0.4	0.4	0.0	0.0(4)	0.0(1)
II.	0.0	0.0	1.2	0.0(4)	-
III.	0.4	0.0	0.0	0.5(4)	2.0(1)
IV.	0.0	0.4	0.0	0.0(4)	2.0(1)
V.	0.0	0.0	0.5	0.0(4)	-
VI.	0.4	0.0	0.4	0.0(4)	2.0(1)
VII.	0.8	0.0	0.0	1.0(2)	2.0(1)
VIII.	0.8	0.8	0.4	0.0(4)	2.0(1)
All groups	.35	.2	.325	.133	1.66

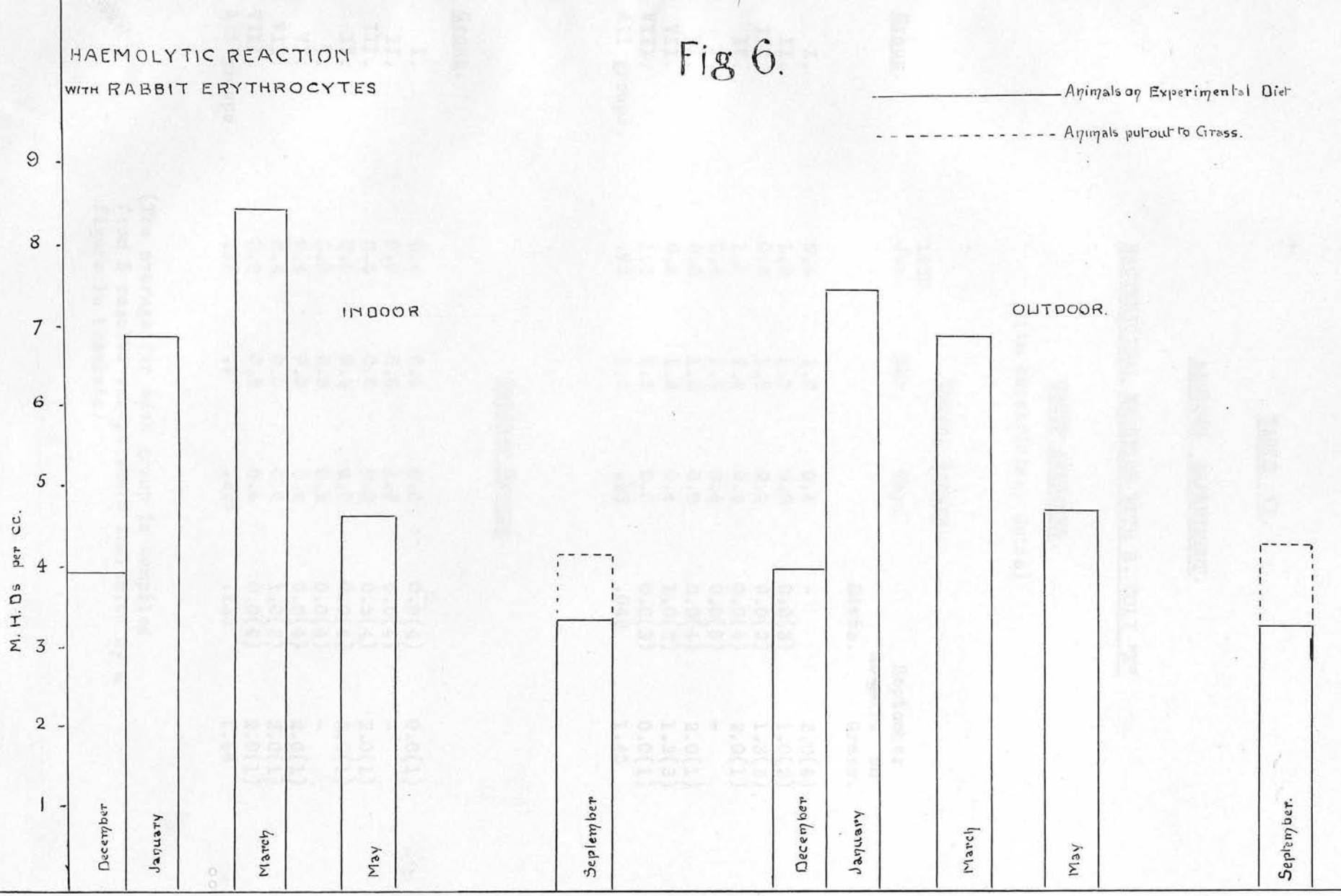
(The average for each group is compiled from 5 results except where indicated by a figure in brackets).

Fig 6.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES

——————— Animals on Experimental Diet

- - - - - - - - - - - - - Animals put out to Grass.



little difference showing between groups I to VI, while groups VII and VIII were markedly higher. In May and September there was no differentiation. Again in September somewhat higher figures were obtained from the animals on grass than from those still on experimental diet.

The peak of the curve of variation from month to month falls in these groups in January. Thereafter a progressive decrease in value occurs. This constitutes a difference between the outdoor and indoor groups of the experiment, which corresponds to that occurring in the bacteriolysis of B.coli "X" by these sera (fig.6).

Agglutination of B. paratyphosus B.
Table VIII.

Indoor Groups. Very little differentiation occurred with this reaction until September. Group IV gave the highest value in December, January and March, then dropped to lowest in May, and was intermediate in September. Group III showed a progressive diminution in value from January to September. In this it differed from all other groups, in which the falling off continued until May only, being succeeded by a rise in September. The average for the animals on grass was very slightly lower than for those on experiment - 5.4 as against 5.8. A rise in the level of the reaction occurred in January as compared with December followed by a fall through March to May and a final rise in September.

Outdoor Groups. As in the indoor groups no differentiation occurred and no consistent behaviour was shown by any group. The difference between the sheep on grass and on the experiment, and the monthly variation are also the same as in the indoor groups.

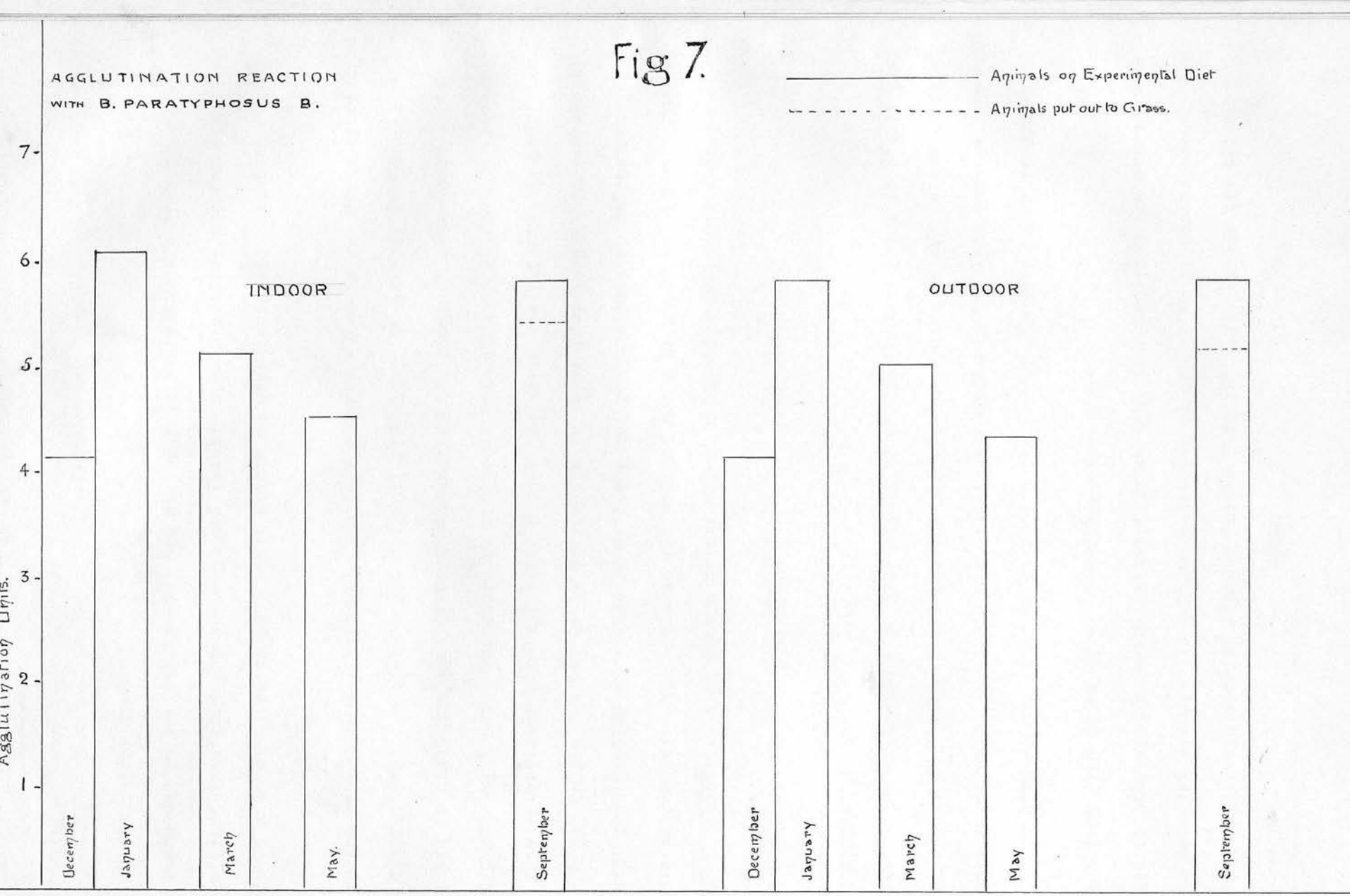
No/

Fig 7.

AGGLUTINATION REACTION
WITH B. PARATYPHOSUS B.

——— Animals on Experimental Diet

- - - - - - - - - - - - - - - Animals put out to Grass.



No difference appears between indoor and outdoor groups (fig.7)

Agglutination of B. abortus (Hog).
Table IX.

There is no record of this test until January, as it was not included among those carried out in December.

Indoor Groups. Little differentiation occurred among these groups. In January group IV was highest, and at all other times it was lowest. Group VI occupied an intermediate position in January and March, and was highest in May and September. The different groups show different curves for the month to month readings. Groups II, III and VI increased from January to May, the latter remaining constant in September, while groups II and III fell slightly in that month. Groups I, IV, V, VII and VIII rose to a maximum in March, falling somewhat in May. Thereafter their behaviour varied, IV and V continuing to fall, VIII remaining steady and VII rising slightly. In March groups I - IV tended to be slightly lower, if anything, than groups V - VIII.

The average of the animals on grass (5.3) is somewhat lower than that of the experimental animals (6.1).

Outdoor Groups. Little variation occurred between the averages in this test. Group II remained remarkably constant throughout the experiment. The groups behaved alike from month to month in that they all reached their highest activity in March. Group I rose again to its March value in September. At that time Groups IV, VI, VII, and VIII gave somewhat lower values than the others, but as with the bactericidal reaction with B.suipestifer this/

TABLE VII.

ASHTOWN EXPERIMENT.

HAEMOLYTIC REACTION WITH RABBIT ERYTHROCYTES.

GROUP AVERAGES.

(M.H.D.s per c.c.)

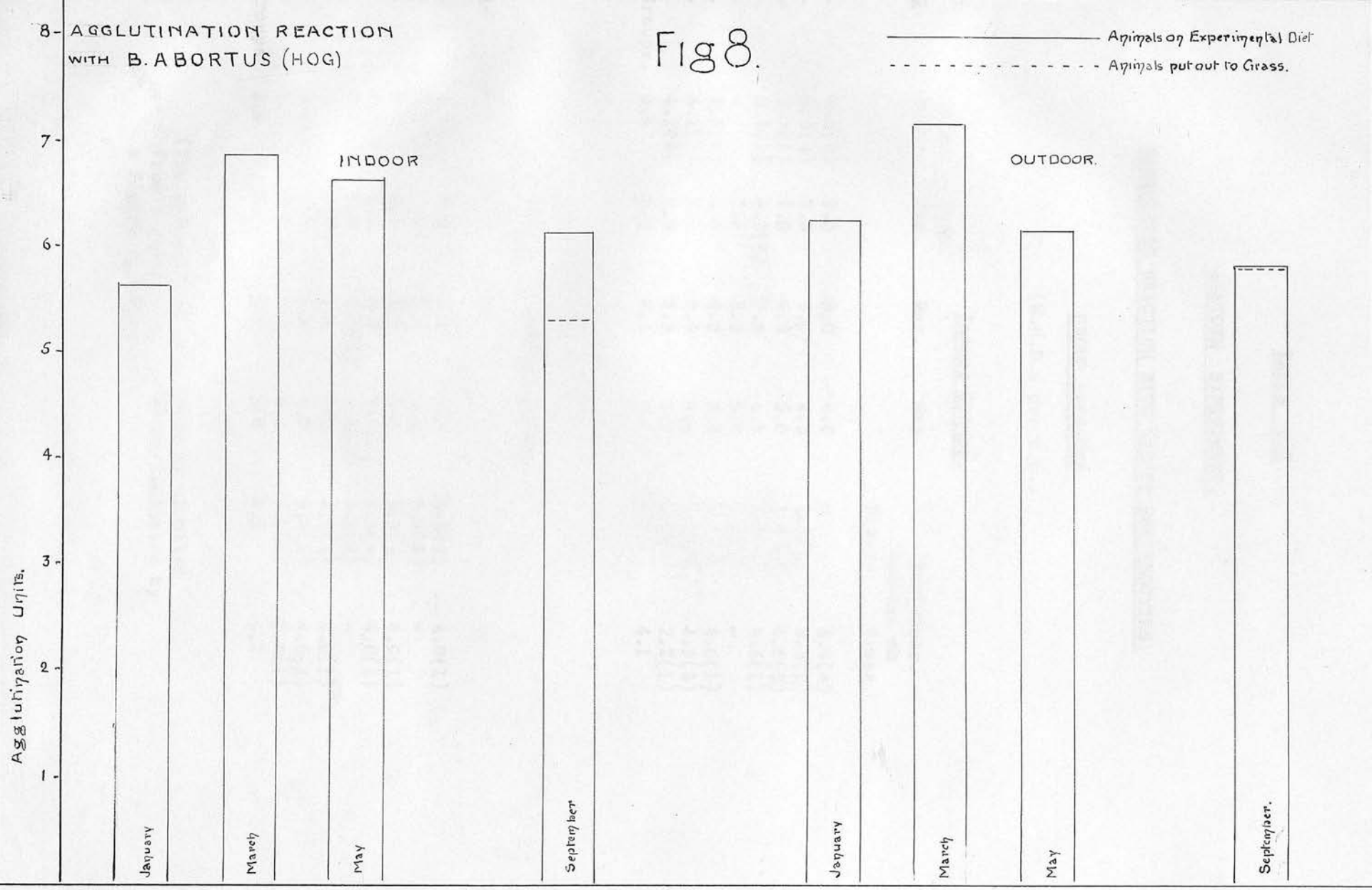
Indoor Groups.

Group.	1929. Dec.	1930. Jan.	Mar.	May.	September. Animals on Diets.	Grass.
I.	3.3(2)	7.5	8.0	4.6	-	3.4(4)
II.	4.2(4)	7.3	8.0	4.3	2.6(3)	5.3(2)
III.	3.3(1)	7.0	9.3	5.0	4.0(2)	4.0(3)
IV.	3.6(2)	4.9(4)	7.6	4.5	3.7(4)	6.6(1)
V.	-	5.6	8.3	5.2	3.2(3)	-
VI.	3.9(2)	6.3	8.3	3.8	3.4(4)	4.0(1)
VII.	4.1(2)	6.6	8.6	4.4	3.4(2)	4.3(3)
VIII.	4.1(4)	8.6	8.8	4.7	3.0(3)	2.2(1)
All Groups.	3.9	6.8	8.4	4.6	3.3	4.1

Outdoor Groups.

Group.						
I.	3.7(3)	7.3	6.6	4.6	2.8(4)	4.0(1)
II.	3.0(2)	8.0	6.6	4.5	4.2(4)	-
III.	4.2(3)	8.1	6.3	4.9	3.2(4)	4.0(1)
IV.	3.6(2)	8.3	6.3	4.7(4)	2.5(4)	4.0(1)
V.	3.9(3)	6.3	6.2(4)	4.6(3)	3.3(4)	-
VI.	-	6.3	6.6	4.6	2.9(4)	4.0(1)
VII.	3.9(3)	7.0	7.3	5.0	3.4(2)	4.0(1)
VIII.	4.7(3)	7.7	8.3	4.3	3.5(4)	5.0(1)
All groups	3.9	7.4	6.8	4.6	3.2	4.2

(The average for each group is compiled from 5 results except where indicated by a figure in brackets).


AGGLUTINATION REACTION
WITH B. ABORTUS (HOG)
Fig 8.
Animals on Experimental Diet
Animals put out to Grass.
INDOOR
OUTDOOR.
Agglutination Units.
8
7
6
5
4
3
2
1
January
March
May
September
January
March
May
September.

this was probably due to sampling on different days.

In comparing the levels of the indoor and outdoor groups it is noticeable that in January and March the outdoor animals are somewhat higher than those indoor, while in May and September, the positions are reversed (fig.8).

Summary.

The complexity of the results makes it difficult to interpret them in the present state of knowledge. The small number of animals in each group also reduces the significance of any differences elicited. Further, all individuals in one group did not behave similarly and groups did not maintain consistent positions relative to one another from one sampling to the next. Where indications of a change might occur at one sampling, these seldom continued until the following time of testing. The results, while in some cases suggestive of correlation between serum reactions and nutritional state, afford no conclusive evidence that the natural antibody content of serum is influenced by the particular nutritional factors concerned in this experiment.

The most noticeable differences in reaction are those occurring:-

(1) between one sampling and another
(2) between animals under indoor and outdoor conditions
(3) between animals on experimental diet and those on grass.

The first two of these appear to be interdependent as the outdoor and indoor groups show maximum strength in two of the reactions at different times. This may possibly be due to the effect of ultra-violet light. It occurs in the bactericidal reaction with B.coli "X" and the haemolytic reaction with rabbit erythrocytes. In both cases the curves of the indoor groups have a peak in March, by which time those of the outdoor groups have already passed

TABLE VIII.

ASHTOWN EXPERIMENT.

AGGLUTINATION REACTION WITH B. PARATYPHOSUS B.

GROUP AVERAGES.

(in agglutination units)

Indoor Groups.

Group.	1929. Dec.	1930. Jan.	Mar.	May.	September. Animals on Diets.	Animals on Grass.
I.	4.0(4)	6.0	5.0	4.4	-	6.0(4)
II.	4.2(4)	5.6	5.6	5.0	5.0(3)	4.5(2)
III.	4.0	6.0	5.0	4.6	4.0(2)	4.3(3)
IV.	4.3(3)	6.4	5.8	4.0	6.0(4)	6.0(1)
V.	4.0(3)	5.6	5.0	4.0	7.0(3)	-
VI.	4.0(3)	5.4	5.0	4.8	6.0(4)	6.0(1)
VII.	3.5(2)	6.0	5.0	4.8	5.5(2)	5.6(3)
VIII.	4.2(4)	5.8	4.8	4.4	6.3(3)	6.0(1)
All Groups.	4.1	6.1	5.1	4.5	5.8	5.4

Outdoor Groups.

Group.						
I.	4.2(4)	5.6	4.8	5.0	5.5(4)	6.0(1)
II.	4.0(4)	5.8	4.4	4.4	5.0(4)	-
III.	3.7(4)	6.5(4)	5.2	4.2	5.5(4)	6.0(1)
IV.	4.0(3)	5.2	5.0	4.0(4)	6.2(4)	5.0(1)
V.	3.7(4)	5.0	5.5	4.6(3)	6.0(4)	-
VI.	5.0(2)	5.6	5.2	4.2	6.2(4)	5.0(1)
VII.	4.5(4)	5.2	4.6	4.6	6.0(2)	5.0(1)
VIII.	3.7(4)	6.2	5.0	3.7(4)	5.7(4)	4.0(1)
All groups.	4.1	5.8	5.0	4.3	5.8	5.1

(The average for each group is compiled from 5 results except where indicated by a figure in brackets).

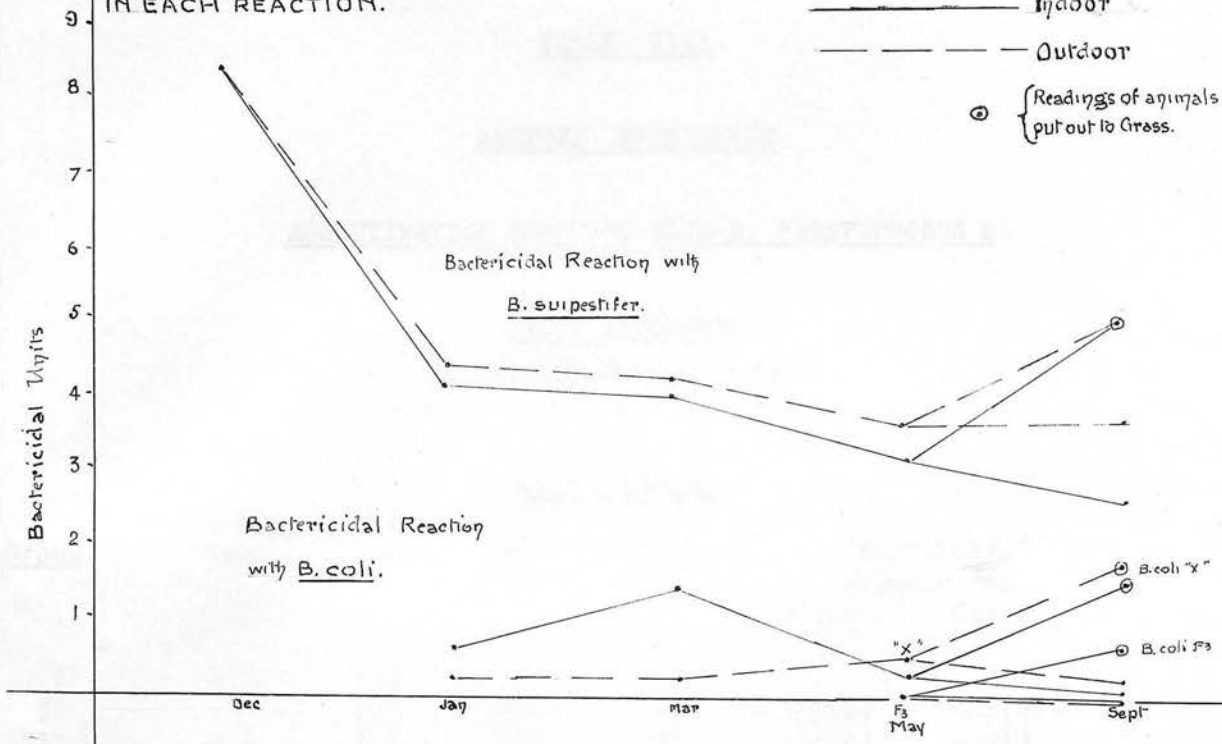
COMPARISON OF RESULTS
FROM INDOOR AND OUTDOOR ANIMALS
IN EACH REACTION.

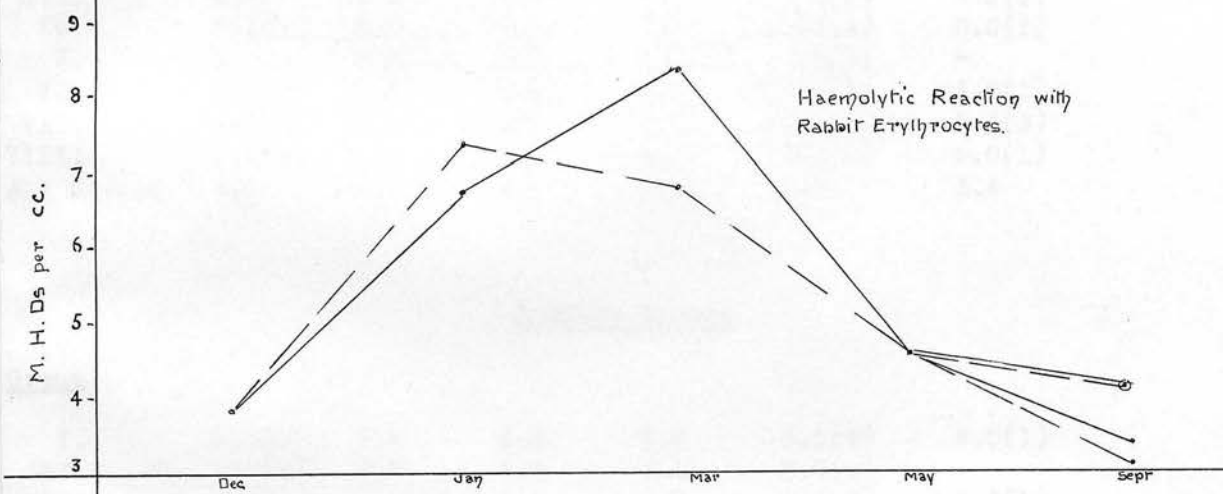
Fig 9.

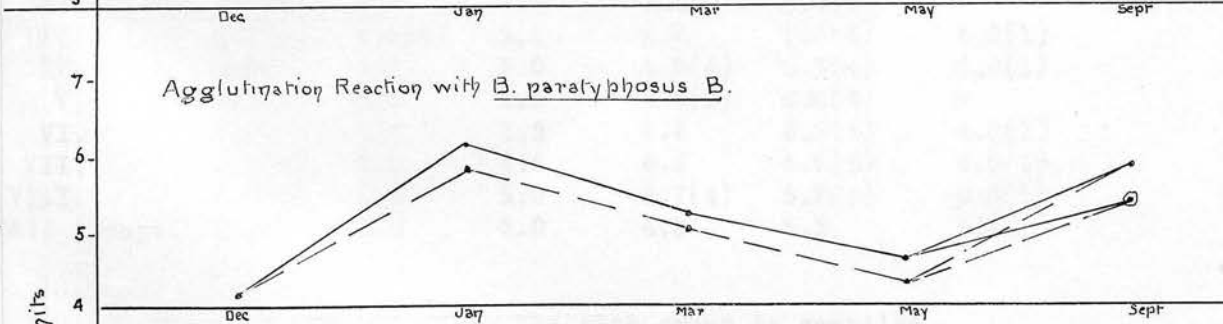
Indoor

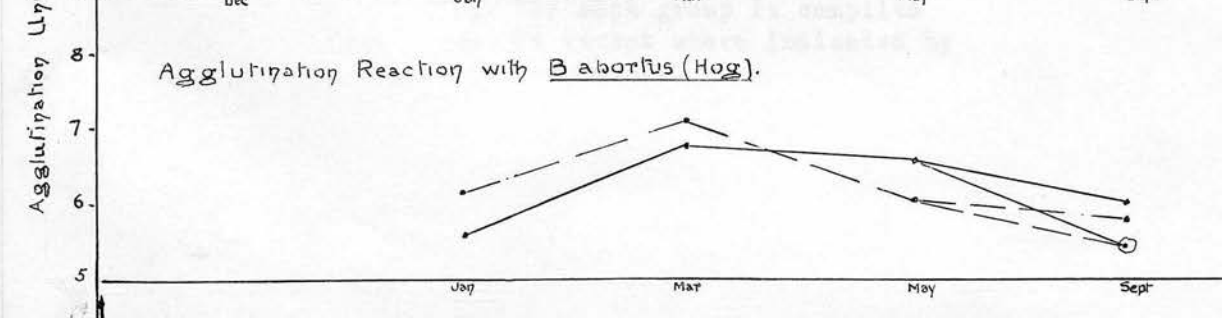
Outdoor

Readings of animals put out to Grass.









their maximum (see Fig.9). The curves for the indoor and outdoor groups for the bactericidal reaction with B. suipestifer and the agglutination with B. paratyphosus B. run remarkably parallel, though in the former reaction the indoor curve tends to drop more rapidly than the outdoor one. In the agglutination reaction with B. abortus (Hog) the two curves exchange their positions, the indoor one becoming the higher. The average figures for all animals, indoor and outdoor, at the different periods of sampling are shown in table X and fig.9.

The above table and figure also include the results of the animals put out to grass, and comparison of those with the results of the experimental animals in September, makes it obvious that some factor has caused an alteration in the antibody content of their sera. It is not possible to define that factor, as not only did a change in feeding occur, but the animals were liberated from the unnatural penned conditions under which they had been living throughout the experiment. Such conditions may have been responsible in part for the steady decline in the strength of all reactions in the latter months of sampling. Only in the agglutination reaction with B. paratyphosus B. did the curve rise at the final testing.

The result of putting animals to grass is different in the case of the lytic and the agglutination reactions. In the former case, such treatment is followed by increased activity of the sera, and in the latter case, by the opposite effect. It will be shown later that these differences between the animals on grass and those on experimental diets, though small, are statistically significant, as are the majority of the differences between the indoor and outdoor animals.

Clinical/

TABLE IX.

ASHTOWN EXPERIMENT.

AGGLUTINATION REACTION WITH B. ABORTUS (HOG).

GROUP AVERAGES.

(in agglutination units)

Indoor Groups.

Group.	1930. Jan.	Mar.	May.	September. Animals Diets.	September. on Grass.
I.	5.4	6.8	6.3(3)	-	5.5(4)
II.	5.8	6.6	7.0(4)	6.5(2)	6.0(2)
III.	5.4	6.4	7.0(4)	6.5(2)	4.3(3)
IV.	6.0	6.4	5.7(4)	5.0(4)	6.0(1)
V.	5.8	7.2	7.0(4)	5.6(3)	-
VI.	5.6	6.6	7.2(4)	7.2(4)	5.0(1)
VII.	5.8	7.2	6.2(4)	6.5(2)	5.6(3)
VIII.	5.4	7.0	6.0(3)	6.0(3)	5.0(1)
All Groups.	5.6	6.8	6.6	6.1	5.3

Outdoor Groups.

Group.					
I.	6.2	7.2	5.5(4)	7.2(4)	8.0(1)
II.	6.2	6.6	6.6(3)	6.5(4)	-
III.	5.8	7.6	6.2(4)	6.5(4)	6.0(1)
IV.	6.4	6.8	6.5(4)	5.5(4)	4.0(1)
V.	6.2	7.7(4)	6.0(3)	6.2(4)	-
VI.	6.2	6.8	6.0(4)	5.0(4)	5.0(1)
VII.	6.4	6.8	6.0(4)	4.0(2)	6.0(1)
VIII.	6.0	7.2	5.7(4)	4.7(4)	6.0(1)
All Groups.	6.2	7.1	6.1	5.8	5.8

(The average for each group is compiled from 5 results except where indicated by a figure in brackets).

Clinical observations were made throughout the experiment, and from these it appeared that, while the diets of groups IV and VIII definitely maintained the sheep in the best conditions, those of groups I and V, after a certain time, were not sufficient to maintain life and the sheep lost weight and fell into poor condition. This difference was not so marked in the outdoor as the indoor sheep and became most evident after April.

TABLE X.

Comparison of Indoor and Outdoor Groups.

Reaction.	Group.	1930. Dec.	1931. Jan.	Mar.	May.	September.		
							Animals on Diet.	Grass.
Bactericidal reaction with B. suipestifer	Indoor	8.4	4.2	4.1	3.1	2.5	3.2	5.0
	Outdoor	8.4	4.5	4.2	3.6	3.6		
Bactericidal reaction with B. coli "X"	Indoor	-	0.75	1.50	0.25	0.05	0.10	1.52
	Outdoor	-	0.35	0.20	0.32	0.13		
Bactericidal reaction with B.coli "F_3"	Indoor	-	-	-	0.05	0.0	0.0	0.52
	Outdoor	-	-	-	0.0	0.0		
Haemolytic reaction with Rabbit Erythrocytes.	Indoor	3.9	6.8	8.4	4.6	3.3	3.3	4.1
	Outdoor	3.9	7.4	6.8	4.6	3.2		
Agglutination reaction with B.paratyphosus B.	Indoor	4.1	6.1	5.1	4.5	5.8	5.8	5.3
	Outdoor	4.1	5.8	5.0	4.3	5.8		
Agglutination reaction with B.abortus (Hog)	Indoor	-	5.6	6.8	6.6	6.1	6.0	5.5
	Outdoor	-	6.2	7.1	6.1	5.8		

SECTION III.

STATISTICAL SURVEY OF THE RESULTS OF THE ASHTOWN EXPERIMENT.

In carrying out the serological tests under consideration, observations were made in the usual manner with a graduated series of quantities of serum or of bacterial emulsion as the case might be, and the lowest dilution with which the complete reaction occurred was recorded. A frequency distribution for each group of animals was thus obtained with regard to each test. The problem at issue was to find whether the frequency distribution of one group of animals under one set of conditions differed significantly from the frequency distribution of another group under a different set of conditions. In order to simplify calculation, it was determined to make an arbitrary division at some point more or less central in respect to each of the two distributions, and to record the number of animals which reacted at dilutions less than this value, and those which reacted at dilutions equal to or greater than this value. The table of comparison so obtained was a fourfold one of the form:

	Reacting at values		
	less than X	equal to or greater than X	Totals
Group 1	a	b	a + b
Group 2	c	d	c + d
	a + c	b + d	a + b + c + d

From this a value χ^2 is deduced such that

$$\chi^2 = \frac{(a+b+c+d)(ad-bc)^2}{(a+b)(c+d)(a+c)(b+d)}$$

From the value of χ^2 so obtained one finds

$$P = \sqrt{\frac{2}{\pi}} \int_{\chi}^{\infty} e^{-\frac{1}{2}\chi^2} \, d\chi$$

which is the probability that an equal or greater difference than that obtained would /

would be likely to occur in a population which was admittedly homogeneous, that is to say that Group 1, and Group 2 did not in fact differ from each other. If, for example, P = 0.01, a difference as great or greater than the observed would be likely to occur once in every 100 sets of observations. It is usually safe to draw a conventional line at P = 0.05, and to consider that any lesser value indicates a real discrepancy. A table giving the values of P for serial values of χ^2 is given by Yule (1922). In the sequel when a value of P equal to or less than 0.05 is found the results will be described as being "statistically significant", but it must always be remembered that this term has no absolute meaning.

1. The first point was the difference between outdoor and indoor conditions (as described on Page 30) at the different seasons of the year when observations were made. The values of P obtained are as follows:-

Table XI.

Values of P obtained by comparison of indoor and outdoor animals at the various periods of sampling.

Month.	Haemolytic Reaction.	Bactericidal Reaction with B.suipestifer.	Bactericidal Reaction with B.coli "X".	Agglutination reaction with B.paratyphosus B.	Agglutination reaction with B. abortus
Dec.	B 0.76	0.8		A 0.38	
Jan.	B 0.34	B 0.32	A 0.27	A 0.43	B 0.03
Mar.	A 0.004 X	B 0.39	A <0.001 X	B 0.10	B 0.05
May.	B 0.34	B 0.02 X	B 0.68	0.45	A 0.007 X
Sept.	A 0.43	B <0.001 X	B 0.74	A 0.71	A 0.47

X indicates a significant result.
A indicates that higher results were obtained from the indoor animals.
B indicates that higher results were obtained from the outdoor animals.

It will be seen that significant differences were observed as regards the haemolytic reaction and bactericidal reaction with B. coli in March, but not at any of the other seasonal periods, and that in both instances the difference was/

was in favour of outdoor conditions. In the case of the agglutination reaction with B. abortus significant differences were observed at the three periods, January, March and May, and in this instance outdoor conditions were favoured at the earlier seasons, and indoor in the later. Agglutination with B. paratyphosus B.did not reveal significant differences at any of the periods. In the bactericidal reaction with B. suipestifer there appeared to be a difference in favour of outdoor conditions which became increasingly significant as the year advanced.

2. The next point investigated was the effect shown in September of putting the animals out to grass. It will be remembered that in June certain animals, which appeared to be on the verge of complete breakdown, were put out to grass, whilst others which were less affected remained under the conditions of experiment. The values of P for the various reactions for animals now under outdoor and indoor conditions, and for both sets combined are as follows:-

Table XII.

Values of P obtained by comparison in September of animals on experimental diets and those put out to grass.

	Haemolytic Reaction.	Bactericidal Reaction with B. suipestifer.	B. coli "X".	Agglutination Reaction wit B.paratyphosus B.	B.abortu
A.	0.08	< 0.001 X	<0.001 X	0.47	0.002 X
B.	0.0045 X	< 0.001 X	<0.001 X	0.12	0.06
A.& B.	0.0006 X	< 0.001 X	<0.001 X	0.19	0.009 X

A = indoor animals.
B = outdoor animals.
X = indicates a significant result.

In the haemolytic and bactericidal reactions the results were higher in the case of the animals on grass than in that of the animals on experimental diet. This was reversed in the agglutination reactions.

It/

It will be seen that the effect of putting the sheep out to grass was the development of significant variations which were revealed by all of the reactions except agglutination with B.paratyphosus B.and in the case of the indoor groups the haemolytic reaction.

3. In estimating the effect of the various diets detailed on page 20, it was considered advisable to examine the effect of each component separately. Thus all the animals which received cod liver oil, were compared with all the animals which did not receive it. The same procedure was followed with regard to protein and calcium. Finally, cod liver oil + calcium was compared with no cod liver oil + calcium. In each case animals under outdoor and under indoor conditions were considered separately and also together. The figures below show that in no instance was a significant difference revealed.

Table XIII.

Values of P. obtained on comparison of the various diets.

	Haemolytic Reaction.	Bactericidal reaction with B.suipestifer.	B. coli.	Agglutination reaction with B.paratyphosus B.	B.abortus.
Cod liver oil v. no cod liver oil.					
A.	0.75	insig.	0.52	0.33	0.64
B.		insig.	0.31	0.16	0.12
A. & B.	insig.	insig.	insig.	0.24	0.19
Protein v. no protein.					
A.	0.33	0.11	insig.	0.33	0.23
B.		0.64	insig.	insig.	insig.
A. + B.	0.22	0.46	insig.	0.36	0.19
Calcium v. no calcium.					
A.	0.33	insig.	insig.	0.75	0.43
B.		0.64	0.31	insig.	insig.
A. + B.	0.26	0.74	insig.	0.36	0.63
Cod liver oil + calcium v. no cod liver oil + calcium.					
A.	insig.	insig.	0.88	0.65	0.36
B.		insig.	0.31	0.31	0.25
A. + B.	0.25	insig.	insig.	insig.	0.16

These/

These comparisons were only made in March, as any differences in the results appear to have been most marked at this time.

GARROCHORAN EXPERIMENT.

In this experiment the sheep were living under natural, and, therefore, much less closely controlled conditions than at Ashtown. As explained previously, this farm was typical of the poor hill pasturage of Western Scotland, with its attendant high mortality and low reproduction rates, and stunted growth of the animals. The stock on the farm were Scottish black-faced sheep.

For a year after the farm was acquired, the sheep were kept on their natural pasture with no supplementary feeding (October, 1929-October,1930) and during that time the four natural divisions or hefts were separated by fencing. The sheep on each heft at the time of fencing were left there, no attempt being made at selection. The hefts converge on the farm buildings and are similar in character and exposure to one another. All arable lands and woods are excluded by the fencing, so that the only pasture available to the sheep is hill pasture. Representative samples from the various hefts were found on analysis (Godden, unpublished results) to have a surprising degree of uniformity in their composition. The analysis of the pasture in August is given in table XIV for CaO, P_2O_5, and N_2. The main deficiencies are in calcium and phosphorus.

Table XIV

	Heft I.	Heft II.	Heft III.	Heft IV.
CaO	0.411	0.462	0.377	0.423
P_2O_5	0.483	0.466	0.571	0.520
N_2	2.664	2.737	2.836	2.713

It was found on making a botanical survey that close agreement held as regards the flora of the hefts.

In the early summer of 1930 four samplings were made and in October of that year/

year supplementary feeding was commenced. The native hill pasture was the basal ration and to this was added a mineral mixture, to make good the deficiency in phosphorus and calcium known to be present, and maize, to raise the caloric value of the pasture. These were given separately and together. The mineral mixture was supplemented with cod liver oil in order to stimulate absorption of calcium and phosphorus.

The mineral mixture consisted of:-

3 parts steamed bone flour
2 parts ground limestone
1 part salt.

Heft I received per head per day 0.25 lbs. maize.

Heft II received mineral mixture (1.5 oz. per head per day and cod liver oil 0.5 c.c. per head per day).

Heft III received maize (as heft I) plus mineral mixture and cod liver oil (as heft II).

Heft IV received no supplementary feeding.

These supplements were placed daily in troughs to which the sheep came to feed.

Blood samples were taken at intervals throughout the year from animals of different ages and sex. Each series consisted of eight samplings, made twice weekly over a period of four weeks. Twenty sheep were bled on each occasion. On the first four days ewes were bled, then gimmers, hoggs, barren ewes and hoggs on the following four days. This order was adhered to in the first two series (November, 1930 and January, 1931), and in the third (March, 1931), the gimmers and hoggs were sampled together on the fifth and sixth days and the barren ewes and hoggs on the seventh and eighth days. In the fourth series (May, 1931), the original order was returned to, except that the barren ewes were omitted and on the eighth day hoggs which had been wintered at Aberdeen and returned to Garrochoran were bled. With the exception of the last six days of/

of the third series, five animals from each heft were bled each day. On these six days in the third series only two hefts were sampled each day, ten ewes from each on the third and fourth days of the series, five hoggs and five gimmers * from each on the fifth and sixth days, and five hoggs and five barren ewes on the seventh and eighth days. (Table in Appendix gives dates of bleeding and animals bled).

It was necessary to bring the blood from Garrochoran to Aberdeen or Edinburgh for examination, and this entailed a journey of about six hours duration. Bleeding was commenced at such a time that immediately on finishing, it was possible to undertake this journey with the minimum of delay. The tubes were placed in an insulated box, to which ice was added either on leaving the farm or 1½ - 2 hours later. Immediately on arrival in Aberdeen or Edinburgh the tubes were taken to the laboratory and the tests commenced.

The time of bleeding varied from 4.0 a.m. to 10.15 a.m. and that of receipt from 2.30 p.m. to 10.15 p.m. The treatment after arrival was the same as that in the Ashtown experiment. The tests also were the same, but in the course of the year two others were added. These were bactericidal reaction with Streptococcus haemolyticus and estimation of the complementing activity of the sheep serum. The tests now used were therefore:-

I. Haemolytic reaction with rabbit erythrocytes.

II. Complementing activity of sheep serum with ox corpuscles, using goat v. ox immune body.

III. Bactericidal reaction with B. suipestifer.

IV. " " " B. coli "X"

V. " " " Streptococcus haemolyticus.

VI. /

* Gimmer = female of the second year.

VI. Agglutination reaction with B. paratyphosus B.

VII. " " " B. abortus (Hog)

The bactericidal reaction with Streptococcus haemolyticus was first used in January, 1931 and the titration of complement in the following July, while the bactericidal reaction with B. suipestifer, and the agglutination reaction with B. paratyphosus B. were discarded after the latter date. The alterations in technique referred to on pages 13 and 14 were made in November, 1931 in the bactericidal reactions with B.coli "X" and Streptococcus haemolyticus.

In addition to the above tests, a bactericidal reaction to a second strain (F_3) of B. coli was used during the first few samplings. As the results obtained were, like those of the reaction with B.coli "X", almost entirely negative, this test was not used after October, 1930.

Preliminary Tests.

The results of the preliminary tests in the summer of 1930 are shown in table XV. On two occasions ewes were bled and,on the other two, barren ewes and wethers. It is remarkable how consistent are the results obtained on each occasion. There are individuals which show rather large variations from the mean but these are very few. The general means of the reactions vary on each occasion. These variations are probably due to the fact that there were animals of different age and sex and that some were lactating and others not, and they will be discussed later when the question of various types of animals arises.

Differences due to breed.

On/

On consideration of the results of the preliminary tests a marked difference was noticed between them and those from the sheep at Ashtown. As the Garrochoran animals were black-faced sheep and the latter Border Leicesters the question arose as to whether the difference might be due to breed, and, accordingly, 16 black-faced ewes were examined at Aberdeen. In order to eliminate the effect of lactation these ewes were chosen with lambs as nearly as possible the same age as those of the ewes tested at Garrochoran. Table XVI shows the results obtained.

Table XVI.

Comparison of results obtained from Scottish blackfaced ewes at Ashtown and Garrochoran and Scottish half-bred ewes at Ashtown.

	Haemolytic Reaction.	Bactericidal Reaction with		Agglutination reaction with	
		B. coli "X"	B. coli "F_3"	B.paratyphosus B.	B.abortus.
	M.H.D.s per c.c.	Bactericidal units.		Agglutination units.	
A.	1.34	0	0	2.9	3.7
B.	4.40	0	0.12	5.3	6.3
C.	8.3	0	0	5.0	6.9

A = Blackfaced ewes at Garrochoran (27/5/30)
B = " " " Ashtown (9/6/30)
C = Half-bred " " " (23/6/30)

From this it can be seen that although the above difference does not appear in the bactericidal reactions it is very noticeable in the haemolytic and agglutinating reactions. This indicates that the difference is not due to breed alone. Figures for Border Leicester Ewes are also included in the table and while in the agglutination tests the figures for these and the Aberdeen black-faced ewes are very similar, in the haemolysin test the Border Leicesters give higher figures than the black-faced ewes.

Daily Variations.

When the consecutive testing in monthly periods had been going on for some/

TABLE XV.

GARROCHORAN.

PRELIMINARY EXPERIMENT.

TESTS.

Wethers and Barren Ewes.

7/5/30. No.	Haem. of Rabbit Eryths.	Bacteriolysis. B.coli "X".	Bacteriolysis. B.coli "F_3".	Agglutination. B.para-typhosus B.	Agglutination. B.abortus.	7/7/30. No.	Haem. of Rabbit Eryths.
13	2.2	0	0	3	7		3.3
74	2.5	0	0	3	3		2.0
80	2.2	0	0	3	2		2.5
83	2.2	0	0	3	5	77	2.5
85	2.2	2.0	0	3	4		2.5
94	2.5	0	0	2	7		2.2
111	2.5	0	0	4	8		2.2
173	2.2	0	0	4	5		2.2
246	2.2	0	0	-	5	248	2.5
250	2.2	0	0	3	8		2.5
254	2.2	0	0	4	6		2.2
316	2.5	0	0	3	5		5.0
358	2.2	2.0	0	3	4		2.2
364	2.2	0	0	3	4		2.5
388	1.6	0	0	2	6		1.6
389	1.6	0	0	1	6		2.0
392	1.5	0	0	3	5		2.5
441	1.6	0	0	3	6		2.0
453	2.2	0	0	3	4		2.5
455	5.0	0	0	3	6		2.5
460	2.2	0	0	2	7		2.5
Averages.							
Wethers.	1.9	0	0	2.5	6.3		2.2
Barren Ewes.	2.4	0.2	0	3.1	5.0		2.6

Bacteriolysis. B.coli "X".	Bacteriolysis. B.coli "F_3"	Bacteriolysis. B.suip-pestifer.	Agglutination. B.para-typhosus B.	Agglutination. B.abortus.	27/5/30. No.	Haem. of Rabbit Eryths.
0	0	4.0	6	8	35	1.1
0	0	4.0	6	7	49	1.2
0	0	2.0	6	7	40	1.1
0	0	2.0	7	8	43	1.1
0	0	2.0	7	7	156	1.3
0	0	2.0	5	8	132	1.2
0	0	4.0	4	7	126	1.2
0	0	4.0	4	6	176	1.5
0	0	2.0	5	8	177	1.6
0	0	4.0	6	6	221	1.3
0	0	4.0	4	6	295	1.5
0	0	4.0	6	7	297	1.3
0	0	4.0	5	7	313	1.5
0	0	4.0	5	6	330	1.3
0	0	4.0	5	7	334	1.5
0	0	4.0	6	6	395	1.2
0	0	4.0	4	7	404	1.2
0	0	4.0	5	6	411	1.2
0	0	4.0	5	7	435	1.6
0	0	2.0	5	6	438	1.5
0	0	2.0	6	6		
0	0	3.6	5.1	6.3	Ewes	1.3
0	0	3.2	5.4	7.0		

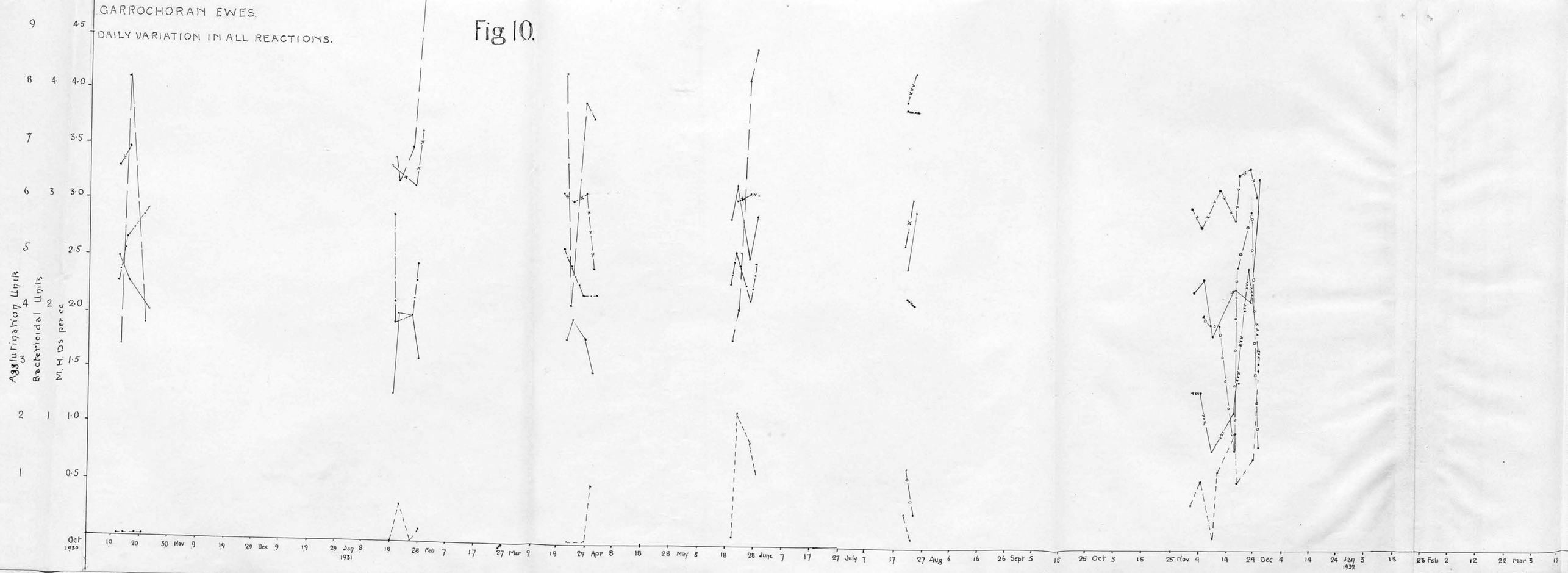
15/7/30.

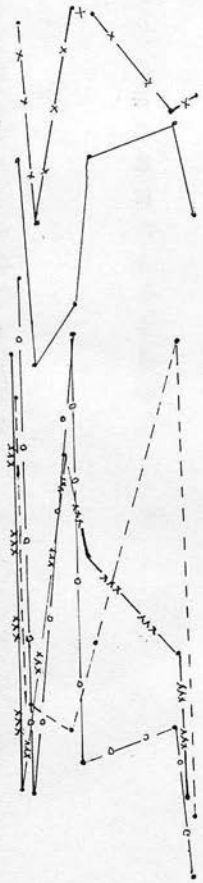
Bacteriolysis		Agglutination.		No.	Haem. of Rabbit Eryths.	Bacter-	:iolysis		Agglutination.	
B.coli "X".	B. coli "F_3".	B.para-typhosus B.	B.abortus.			B. coli "X".	B. coli "F_3".	B.suip-:estifer.	B. para-typhosus B.	B. abortus.
0	0	3	1		2.5	0	0	2.0	6	6
0	0	2	5	67	2.8	0	0	2.0	5	6
0	0	2	3		2.8	0	0	2.0	6	7
0	0	2	5		2,8	0	0	2.0	5	6
0	0	3	3	157	3.3	0	0	2.0	6	6
0	0	2	6		3.3	0	0	2.0	6	7
0	0	3	6	123	2.5	0	0	2.0	5	5
0	0	3	5		2.5	0	0	2.0	7	7
0	0	3	1		4.0	0	0	2.0	7	6
0	0	3	3		3.3	0	0	2.0	5	7
0	0	4	4		2.8	0	0	2.0	7	7
0	0	3	3		2.8	0	0	2.0	6	7
0	0	4	4		2.8	0	0	2.0	7	7
0	0	3	3		2.8	0	0	2.0	6	7
0	0	2	3		2.5	0	0	2.0	3	6
0	0	4	3		3.3	0	0	2.0	5	6
0	0	4	1		2.8	0	0	2.0	5	6
0	0	3	4		2.5	0	0	2.0	3	7
0	0	3	3		2.5	0	0	2.0	4	6
0	0	3	5		2.0	0	0	2.0	3	6
0	0	4	4		4.0	0	0	2.0	5	6
0	0	2	4		3.3	0	0	2.0	5	6
				(269	3.3	0	0	2.0	6	6)
0	0	2.9	3.6		2.9	0	0	2.0	5.2	6.2

GARROCHORAN EWES.

DAILY VARIATION IN ALL REACTIONS.

Fig 10.





———————————— Haemolylic Reaction.

——— ——— ——— Bactericidal Reaction with B. suipestifer.

- - - - - - - - - - - - Bactericidal Reaction with B. coli "X".

——— o ——— o ——— Bactericidal Reaction with Streptococcus Haemolyticus

——— • ——— • ——— Agglutination Reaction with B. paratyphosus B.

——— x ——— x ——— Agglutination Reaction with B. abortus (Hog).

——— xxx ——— xxx ——— Titration of Complement.

23 Apr 2 12 22 May 2 12

some time it was noticed that there were definite variations in the results obtained from day to day and that these differences affected all animals. This suggested that the discrepancy might be due to some fault in the technique of the tests whereby variations in the standards and conditions occurred. Great care has been taken throughout to keep these as uniform as possible, and the fact that changes occurred in all tests points to some other factor or factors being responsible for the variations.

Ewes. Figure 10 and Table XVII show the extent to which the mean value for each reaction varied from sampling to sampling, while from Tables XX - XXVI it may be seen that all hefts behaved similarly.

The greatest variations occurred in the bactericidal reactions, that with B. suipestifer showing a difference of as much as 2.4 units between two samplings, one two days later than the other. In each period when this test was in use it showed a variation almost as great as this.

Little variation occurred at first in the reaction with B. coli "X", as this gave consistently negative results. After the change in technique in November, 1931, however, larger variations appeared, e.g. the mean value of the reaction on April, 11th, 1932 was 2.4 units, and on April, 13th 0.8 units.

The bactericidal reaction with Streptococcus haemolyticus showed variations of the same order as the reaction with B. suipestifer, e.g. on November 23rd the mean activity was 3.0, and on November 25th 0.9 units.

The haemolytic reaction with rabbit erythrocytes gave results which maintained fairly steady levels within each period, but occasional marked divergences appeared

In/

In May,1931 for example, the mean figures on the four consecutive samplings (21st, 23rd, 27th and 29th) were 2.9, 3.2, 2.5 and 2.9 M.H.D.s per c.c. Though these fall within quite a small compass, yet the limits of that compass were attained on two consecutive days. Again, on 23rd and 25th November, 1931, the mean number of M.H.D.s per c.c. was 2.2 and 3.3 respectively.

The complement titre remained, on the whole, remarkably steady, though on the 21st, 23rd and 28th March, 1932, mean values of 11.8, 4.6, and 10.0 M.H.D.s per c.c. were obtained.

On only one occasion did the mean of the agglutination reaction with B. paratyphosus B. vary by more than one unit on two consecutive samplings, This one variation was from 5.8 units on 19th January, 1931 to 3.9 units on 21st January.

The agglutination reaction with B. abortus has shown greater variation in the last two periods of sampling, i.e. November, 1931 and March, 1932, than at any previous time. From October, 1930 to July, 1931 the variation between two consecutive samplings never exceeded 1 unit. Subsequent to that variations reached a maximum of 1.7 units.

The greatest variation occurring in each reaction during any period of sampling is as follows:-

		Date.
Haemolytic Reaction with rabbit erythrocytes	1.4 M.H.D.s per c.c.	Nov.1931.
Titration of Complement	8.3 "	Nov.1931.
Bactericidal reaction with B.suipestifer	2.7 units.	Jan.1931.
Bactericidal reaction with B. coli "X"	1.6 "	Mar.1931.

Bactericidal/

		Date
Bactericidal reaction with Streptococcus haemolyticus	2.1 units	Nov. 1931.
Agglutination reaction with B. paratyphosus B.	1.9 units	Jan. 1931.
Agglutination reaction with B. abortus	1.7 units	Nov. 1931.

The greater divergence of results in November, 1931 is probably attributable to the greater number of samplings at this time, since ewes were sampled on all eight days.

Hoggs. Whereas there were usually four samplings from ewes in each period, there were only two from hoggs. In April, 1931, however, there were four samplings, two hefts being taken on each occasion (vide p.39). The question arises as to whether these figures for April may be taken into account, but, as it has already been pointed out that these daily variations affect all hefts similarly (though not equally), it seems possible to consider them with the other results. It must be borne in mind, however, that this was the time at which differences between the hefts were most marked.

In the haemolytic reaction with rabbit erythrocytes the results are remarkably uniform from day to day, the greatest difference being 0.4 M.H.D.s per c.c. between 8th and 13th April, and 0.5 M.H.D.s per c.c. for the whole of that period.

The bactericidal reactions show divergences similar to those occurring with ewes' sera, e.g. the differences between 4th and 11th February in the reactions with B. suipestifer and B. coli "X" were 1.1 and 1.0 units respectively, and between 6th and 8th April in the reactions with B. suipestifer and Streptococcus haemolyticus were 1.9 and 2.0 units respectively.

The/

Group. B.

—xxx— xxx— Titration of Complement

—o— o— Bactericidal Reaction with Streptococcus Haemolyticus.

—x— x— Agglutination Reaction with Baborius (Hog)

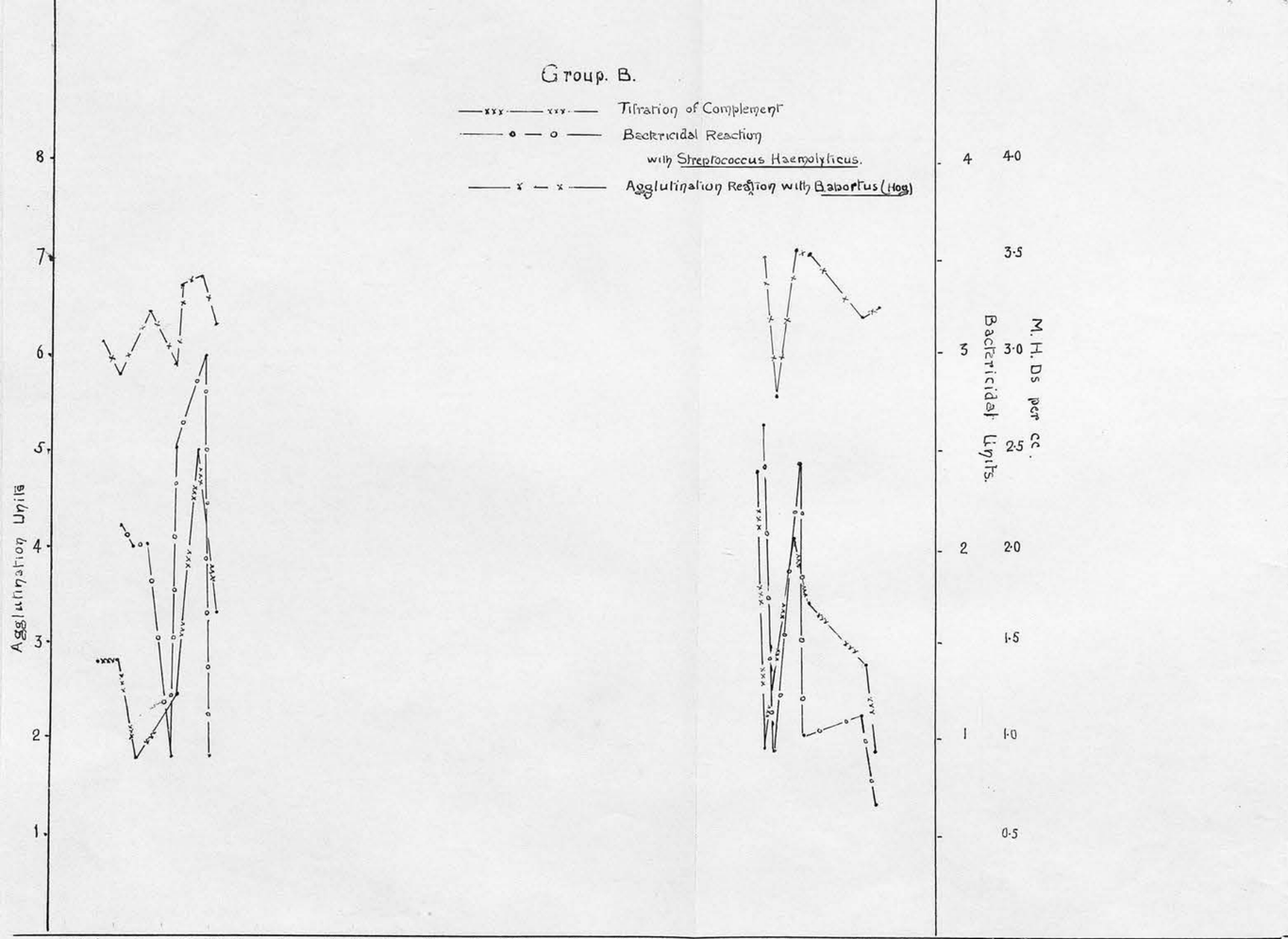


Fig 11.

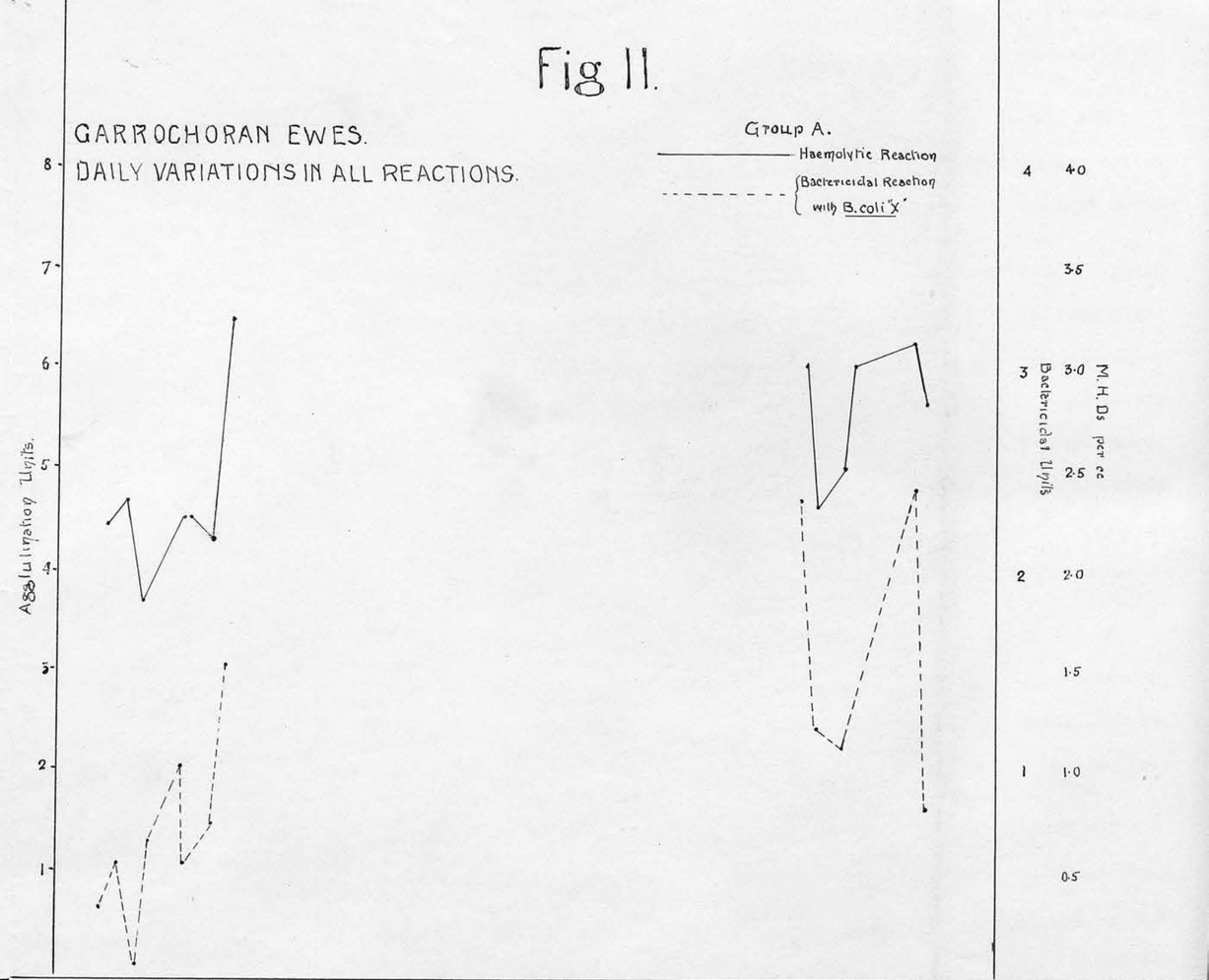
GARROCHORAN EWES.

DAILY VARIATIONS IN ALL REACTIONS.

Group A.

——— Haemolytic Reaction

- - - - - - - - Bactericidal Reaction with B. coli "X"



The variations in the agglutination reactions were also similar in degree to those seen among the results with ewes' sera.

Figure 10 makes obvious the difficulty of tracing any parallelism between the reactions. Indeed from October, 1930 until July, 1931, it almost appears as though every permutation of rise and fall had gone to the making of the different curves. Closer inspection, however, shows that in January, 1931 the curves of the reactions with B. suipestifer, B. paratyphosus B., and B. abortus exhibit a certain similarity, while that of the haemolytic reaction is in almost direct opposition to them. This similarity is not maintained in the subsequent portions of the curve. In May, 1931 the curves of the reactions with B. paratyphosus B. and rabbit erythrocytes run definitely parallel while that of B. coli reaction behaves similarly to a certain extent. By November, 1931 the tests with B. suipestifer and B. paratyphosus B. had been discarded, and the technique of the bactericidal reactions with B. coli and Streptococcus haemolyticus had been revised and the titration of complement added. The portion of the fig. 10 relating to this time becomes very complicated, but fig. 11 shows how it is possible to separate the reactions at this and the subsequent period into two divisions, in each of which the components behave somewhat alike. The grouping is:-

A. Haemolytic reaction with rabbit erythrocytes,
Bactericidal reaction with B. coli "X"

B. Titration of complement,
Bactericidal reaction with Streptococcus haemolyticus,
Agglutination reaction with B. abortus.

It is curious that the complement titre should run parallel to the reactions which are not dependent on complement, rather than to those in which it plays a part.

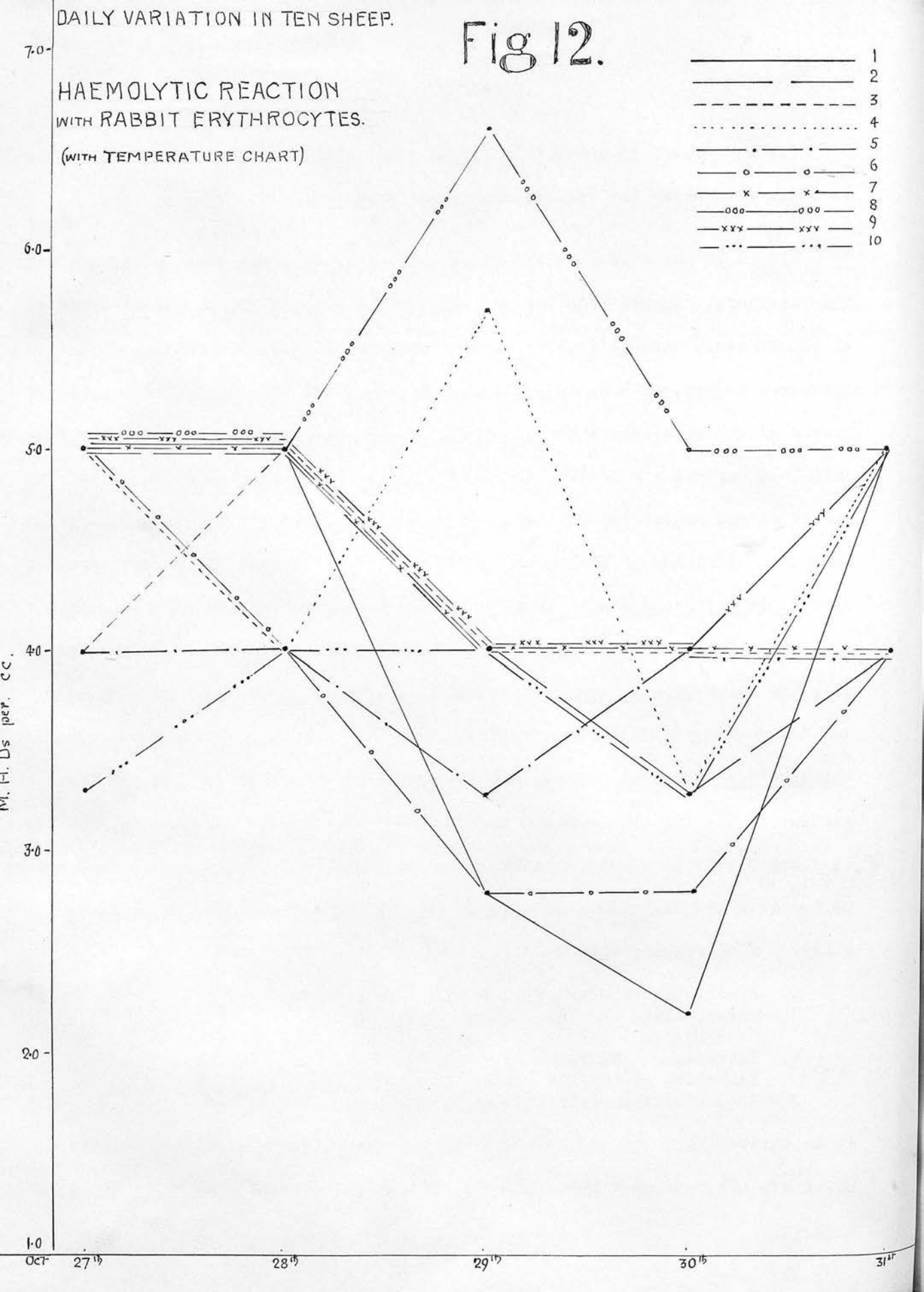
It/

DAILY VARIATION IN TEN SHEEP.

Fig 12.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES.

(WITH TEMPERATURE CHART)



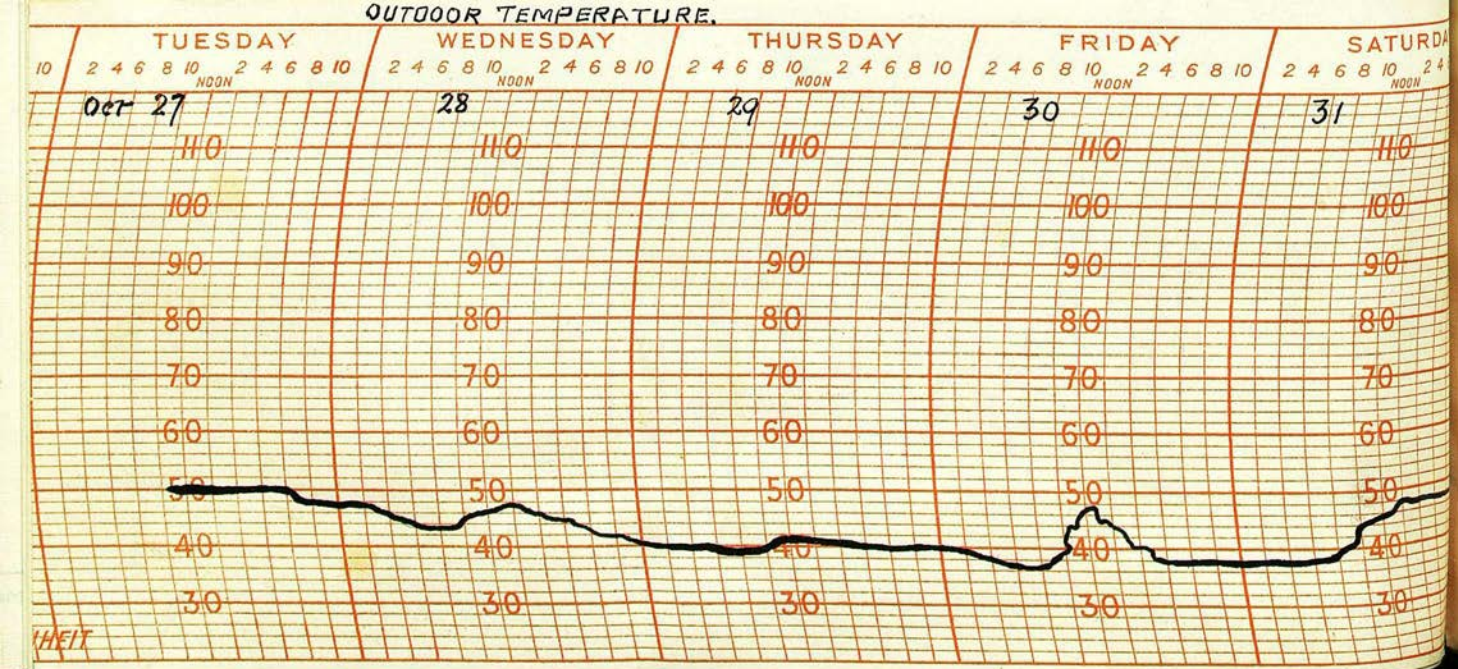
It is noteworthy that all reactions showed a very marked fall in value on March, 23rd, 1932 as compared with the results obtained two days previously. Similarly a small rise occurred in all reactions on 23rd May, 1931 as compared with 21st May, but this is not nearly so marked as the fall in March.

It is this similarity in behaviour among the different reactions which makes it appear that the day-to-day variations are due to some factor or factors affecting the serum, and not to changes in the technique, which would be expected to influence different reactions differently.

In order to try and elucidate what these factors might be, a series of observations were made under controlled conditions. Ten sheep were maintained specially for this purpose. The reaction used was the haemolysis of rabbit erythrocytes as being simple and rapidly carried out. Erythrocytes were obtained from the same rabbit throughout.

The first possibility to be considered was a daily variation of the antibody content of the serum of different animals and the effect on it of meteorological conditions. The animals were therefore bled and the tests

OUTDOOR TEMPERATURE.



It is noteworthy that all reactions showed a very marked fall in value on March, 23rd, 1932 as compared with the results obtained two days previously. Similarly a small rise occurred in all reactions on 23rd May, 1931 as compared with 21st May, but this is not nearly so marked as the fall in March.

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In order to try and elucidate what these factors might be, a series of observations were made under controlled conditions. Ten sheep were maintained specially for this purpose. The reaction used was the haemolysis of rabbit erythrocytes as being simple and rapidly carried out. Erythrocytes were obtained from the same rabbit throughout.

The first possibility to be considered was a daily variation of the antibody content of the serum of different animals and the effect on it of meteorological conditions. The animals were therefore bled and the tests carried out on five consecutive days. During this time the temperature varied from 50°F at the commencement of the observations, to 37°F on the fourth and fifth days, and snow fell on the third day. The results are given in Table XVIII and from the accompanying graph (fig.12) it will be seen that no consistent differences appeared among the individual animals. Consideration of the averages of all animals shows a drop in haemolysin content when the temperature fell, but the rise on the fifth day occurred before the rise in temperature.

The/

The next question which arose was the effect which transport and temperature might have on the haemolysin and other immune bodies in the serum, after withdrawal from the animal. Five samples of blood were therefore taken from each of five animals. These were treated as follows:-

I. Serum separated immediately and divided into 2 portions -
(a) haemolytic reaction tested immediately.
(b) placed in refrigerator for 8 hours.

II. Maintained at room temperature for 8 hours.

III. Placed in refrigerator for 8 hours.

IV. Placed in insulated box, without ice for 4 hours, then ice added for a further period of 4 hours.

V. As IV, but agitated by motor transport throughout.

At the end of 8 hours, serum was separated from samples II - V, and the haemolysin content of these and sample Ib estimated (see Table XVIII). Again no consistent behaviour can be observed among individuals, the changes occurring being hardly beyond those of experimental error, except in sample Ib. Under the treatment afforded to this portion of the blood the haemolysin content of two of the samples dropped considerably, and thus brought down the general average. From the results obtained, however, it is obvious that the conditions of transport of the blood from Garrochoran were not the cause of the daily variations encountered.

Next the effect of:-

(1) being kept overnight in a pen, and
(2) starvation,

were examined, but neither caused any variation in haemolysin content (Table XVIII).

So far, then, no satisfactory explanation has been found to account for the/

TABLE

XVII.

DAILY AVERAGES

FOR ALL REACTIONS.

Ewes.

	1930. May 27.	Jly. 15.	Oct. 13.	15.	22.	1931. Jan. 19.	21.	26.	28.	Mar. 23.	25.	30.	Apl. 1.	May. 21.
Haemolytic Reaction.	1.3	2.9	2.5	2.3	2.0	1.3	2.0	2.0	1.7	1.8	2.0	1.8	1.5	2.9
Titration of Complement.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bactericidal (*B. suipestifer*	-	2.0	1.7	4.1	1.9	3.4	3.2	3.5	5.9	4.2	2.1	3.9	3.8	1.8
reaction with(*B. coli* "X"	0	0	0	0	0	0	0.3	0	0.1	0	0	0	0.5	0.1
(*Str. haemolyticus*	-	-	-	-	-	0.1	0.2	0	0.3	0	0.3	0.5	0	-
Agglutination(*B. paratyphosus* B.	2.9	5.2	4.5	5.3	5.8	5.8	3.9	4.0	4.9	5.2	4.9	4.4	4.4	4.6
reaction with(*B. abortus* (Hog)	3.6	6.2	6.6	6.9	-	6.7	6.5	6.3	7.3	6.2	6.1	6.2	4.9	5.8

Gimmers.

	1930. Oct. 27.	1931. Feb. 2.	Apl. 6.	8.	June. 2
Haemolytic Reaction.	1.9	1.6	2.6	2.1	1.8
Bactericidal (*B. suipestifer*	4.1	3.9	4.1	2.0	4.1
reaction with(*B. coli* "X"	0	0.9	0.2	0	0
(*Str. haemolyticus*	-	0.2	0.5	1.4	0.3
Agglutination(*B. paratyphosus* B.	5.7	4.6	4.0	4.8	4.5
reaction with(*B. abortus* (Hog)	-	3.5	5.2	4.8	6.2

Hoggs.

	1930. Oct. 29.	Nov. 6.	1931. Feb. 4.	Apl. 11.	6.	8.	13.	15.	June. 4.	8.
Haemolytic Reaction.	1.7	1.6	2.4	2.6	2.2	2.3	2.7	2.7	1.9	2.2
Bactericidal (*B. suipestifer*	1.9	1.9	1.7	2.8	3.7	1.8	3.2	4.1	4.3	3.7
reaction with(*B. coli* "X"	0.7	0	0	1.0	0.2	0	0	0.2	0.3	0.4
(*Str. haemolyticus*	-	-	0	0.1	1.0	3.0	0.4	1.3	-	0.7
Agglutination(*B. paratyphosus* B.	5.3	3.1	3.4	4.3	3.3	4.2	3.8	3.4	3.1	3.5
reaction with(*B. abortus* (Hog)	-	6.2	6.0	-	5.8	4.9	6.0	7.1	5.8	5.9

			Jly.		Nov.				
23.	27.	29.	20.	22.	2.	5.	9.	11.	17.
3.2	2.5	2.9	2.5	3.0	2.3	2.4	1.9	-	2.3
-	-	-	19.8	21.2	7.0	6.9	4.3	-	6.0
2.1	4.1	4.4	3.9	3.9	-	-	-	-	-
1.4	0.9	0.6	0.3	0.1	0.4	0.6	0.1	0.7	1.1
0.3	-	0	0.7	0.3	-	2.1	2.0	2.0	0.9
5.2	4.3	5.0	4.4	4.3	-	-	-	-	-
6.1	6.2	6.2	5.4	6.2	6.1	5.8	6.7	7.5	5.9

			1932. Mar.				Apl.	
18.	23.	25.	21.	23.	28.	30.	11.	13.
2.3	2.2	3.3	3.0	2.2	2.5	3.0	3.1	2.8
7.6	12.6	8.2	11.8	4.6	10.0	8.4	6.7	4.4
-	-	-	-	-	-	-	-	-
0.6	0.8	1.6	2.2	1.2	1.1	1.4	2.4	0.8
2.5	3.0	0.9	2.6	0.9	2.4	1.0	1.1	0.6
-	-	-	-	-	-	-	-	-
6.7	6.8	6.3	6.9	5.5	7.0	6.9	6.3	6.4

Barren Ewes.

1930.			1931.		
May	Jly.	Nov.	Feb.	Apl.	
7.	7.	3.	9.	13.	15.
2.4	2.6	1.9	3.4	2.9	2.4
-	3.2	2.5	3.8	4.0	3.7
0.2	0	0	0.6	0	0.5
-	-	-	0.4	0.2	0.8
3.1	5.4	3.9	3.9	3.9	4.0
5.0	7.0	5.4	7.6	6.2	7.5

Results of Haemolytic Reaction and Titration of Complement are given as M.H.D.s per c.c.
" " Bactericidal Reactions are given as bactericidal units.
" " Agglutination Reactions are given as agglutination units.

the great variations occurring from day to day among the Garrochoran samples. It is, however, possible that the Garrochoran sheep, being less well-conditioned than the animals used in the foregoing investigation, might be more susceptible to changes in environment and meteorological conditions, and that this was reflected in the serum content of the natural antibodies. In order to eliminate this daily variation as far as possible when dealing with heft differences, an attempt has been made to take the same number of sera from each heft each day. Where this is done, mean results for each heft may be calculated over a number of days.

The variation from day to day makes it difficult to estimate any seasonal variation, except where this is very marked, and it also complicates the comparison of different types of animals as these were usually sampled on different days.

Seasonal Variation.

This question may be considered here as it is influenced to such an extent by the daily variations. From the figure 10, used in considering the last question, it will be seen that in all reactions, overlapping of the limits of the results obtained occurs from period to period. This overlapping is so great that, except in the haemolytic reaction, it is difficult to say whether any seasonal variation has occurred or not, without statistical analysis, which will be applied in a later section of the thesis.

In the case of the haemolytic reaction with rabbit erythrocytes it may be seen that a decrease in strength occurred from October to January and March, followed in May by a return to a higher level than previously noted. A decrease had occurred again by November with a slight increase in March of the following year/

TABLE XVIII.

Results of trials to find the cause of daily variations in the haemolytic reaction with rabbit erythrocytes.

.....

. Individual variation from day to day. (M.H.D.s per c.c.)

Sheep Number.	Oct.27.	28.	29.	30.	31.
1.	5.0	5.0	2.8	2.2	5.0
2.	5.0	5.0	4.0	3.3	4.0
3.	4.0	5.0	4.0	4.0	4.0
4.	5.0	4.0	5.7	3.3	5.0
5.	4.0	4.0	3.3	4.0	4.0
6.	5.0	4.0	2.8	2.8	4.0
7.	5.0	5.0	4.0	4.0	4.0
8.	5.0	5.0	6.6	5.0	5.0
9.	5.0	5.0	4.0	4.0	5.0
10.	3.3	4.0	4.0	3.3	5.0
Average.	4.6	4.6	4.2	3.6	4.5

I. Effect of varying treatment on the blood after withdrawal from animals. (M.H.D.s per c.c.)

Sheep Number.	Tested Immediately.	Tested 8 hours later. Room Temp.	Refrig-:erator.	Box.	Trans-:port.	Separated.
4.	5.7	5.0	5.0	4.0	5.0	4.0
5.	3.3	2.8	2.8	2.8	3.3	2.2
6.	2.8	2.8	3.3	3.3	3.3	2.8
7.	4.0	4.0	4.0	3.3	3.3	3.3
8.	6.6	5.0	5.0	4.0	5.0	2.8
Average.	4.5	3.9	4.0	3.5	4.0	3.0

(Box = blood kept 4½ hours without ice, then a further 5 hours after the addition of ice to the containing box.
Transport = similarly treated and agitated by transport.
Separated = serum separated immediately and kept in refrigerator).

III. Effect of confinement in pens and starvation.

Sheep Number.	Nov. 30.	Dec.1.	10.	11.	12.
1.	3.0	4.0	-	5.0	5.0
2.	4.0	3.3	5.0	5.0	3.3
3.	5.0	4.0	5.0	5.0	5.0
4.	5.0	5.0	4.0	4.0	4.0
5.	6.6	5.0	4.0	5.0	5.0
Average 1-5	5.1	4.3	4.5	4.8	4.3
6.	3.3	4.0	3.3	3.3	5.0
7.	5.0	4.0	4.0	4.0	4.0
8.	3.3	3.3	3.3	5.0	5.0
9.	4.0	4.0	4.0	5.0	3.3
10.	5.0	4.0	5.0	4.0	3.3
Average 6-10	4.1	3.9	3.9	4.3	4.1

Numbers 1 - 5 acted as controls, being kept in a field and brought into the buildings as required for sampling. Numbers 6 - 10 were kept in an indoor pen between the bleedings on 30th November and 1st December. They were also kept indoors from 9th - 11th December and during that time received neither food nor water. They were returned to the field on 11th December.

year. This increase occurring earlier in 1932 than in 1931 was probably due to the mild winter experienced that year.

Table XIX gives mean values for the reactions over all days in each period, while the seasonal differences of the separate hefts are given in tables (XX - XXVI) and will be considered under the heading "heft differences".

It must be remembered that it is really only heft IV animals which show a true seasonal variation, since in other hefts the effect of supplementary feeding must always be taken into consideration.

The three main problems still to be discussed may be arranged under the headings:-

(1) Individual variations.
(2) Heft differences.
(3) Differences between types of animals.

Individual variation deals with variations occurring among animals sampled at the same time, and so far as is known, similar in regard to age, sex, state of pregnancy or lactation. In the case of the ewes the age is uncertain, all breeding ewes over two years old being grouped together under this title.

Another aspect of "individual variation" is the response of individuals to the tests at different times. Unfortunately, only a few sheep in each heft have been sampled in more than three consecutive periods, but the results from those few will be considered under this heading.

Heft differences implies the difference between the results obtained from sheep in different hefts sampled at the same time. The elucidation of these differences necessitates examination of the mean results from each heft on each day/

day of sampling and for each period, and also the behaviour of each heft from period to period.

Differences between types of animals. This heading includes examination of the results obtained from animals of different age or sex, breeding or barren, and those at different stages or pregnancy and lactation. Here arises the difficulty pointed out when discussing day to day variation, i.e. that these different types of animals were usually tested on different days. The only occasions on which different types were tested together were in April, 1931 when on two days hoggs and gimmers were sampled together and on other two days barren ewes and hoggs were mixed.

The most convenient way of dealing with these three problems is to take each reaction separately and discuss it under the above headings. In this way much unnecessary repetition will be avoided.

Tables XX - XXVI have been prepared for each reaction showing:-

(a) The mean result on each day of sampling,
    (1) for each heft (mean of five results)
    (2) for all hefts together (mean of twenty results)

(b) The mean result for each period of sampling,
    (1) for each heft (mean of twenty results for ewes and of ten for hoggs)
    (2) for all hefts together (mean of eighty results for ewes, and of forty for hoggs).

(Where the number of results from which the mean was compiled differed from those stated above, this is indicated by small figures in brackets following the figure for the mean).

These figures have been calculated for ewes, gimmers, hoggs, and barren ewes. In the case of the gimmers and barren ewes, each period consisted of only one day except in April, 1931, and, therefore, there are no figures for the period means/

TABLE XIX.

PERIOD AVERAGES FOR ALL REACTIONS.

Ewes.

	1930. May.	July.	Oct.	1931. Jan.	Mar.	May.	Jly.	Nov.	1932. Mar.
Haemolytic Reaction.	1.3	2.9	2.2	1.8	1.8	2.9	2.7	2.4	2.8
Titration of Complement.	-	-	-	-	-	-	20.6	7.5	7.7
Bactericidal (B. suipestifer	-	2.0	2.6	4.0	3.4	3.1	3.9	-	-
reaction with(B. coli "X"	0	0	0	0.1	0.1	0.7	0.2	1.5	3.1
(Str. haemolyticus	-	-	-	0.2	0.3	0.2	0.5	1.8	1.5
Agglutination(B. paratyphosus B.	2.9	5.2	5.2	4.6	4.7	4.8	4.3	-	-
reaction with(B. abortus (Hog)	3.6	6.2	6.7	6.7	5.8	6.1	5.8	6.5	6.5

Gimmers.

Haemolytic Reaction.	-	-	1.9	1.6	2.3	1.8	2.4	2.6	1.9	3.4	2.8	-
Titration of Complement.	-	-	-	-	-	-	-	-	-	-	-	-
Bactericidal (B. suipestifer	-	-	4.1	3.9	3.0	4.1	-	3.2	2.5	3.8	3.8	-
reaction with(B. coli "X"	-	-	0	0.9	0.1	0	0.2	0	0	0.6	0.2	-
(Str. haemolyticus	-	-	-	0.2	1.0	0.3	-	-	-	0.4	0.5	-
Agglutination(B. paratyphosus B.	-	-	5.7	4.6	4.4	4.5	3.1	5.4	3.9	3.9	3.9	-
reaction with(B. abortus (Hog)	-	-	-	3.5	5.0	6.2	5.0	7.0	5.4	7.6	6.8	-

Barren Ewes.

Hoggs.

Haemolytic Reaction.	-	-	1.7	2.6	2.6	2.2
Titration of Complement.	-	-	-	-	-	-
Bactericidal (B. suipestifer	-	-	1.9	2.2	3.2	4.0
reaction with(B. coli "X"	-	-	0.6	0.5	0.1	0.3
(Str. haemolyticus	-	-	-	0.1	1.4	0.7
Agglutination(B. paratyphosus B.	-	-	4.2	3.9	3.6	3.3
reaction with(B. abortus (Hog)	-	-	6.2	6.0	6.0	5.9

Results of Haemolytic Reaction and Titration of Complement are given as M.H.D.s per c.c.

" " Bactericidal Reactions are given as bactericidal units.

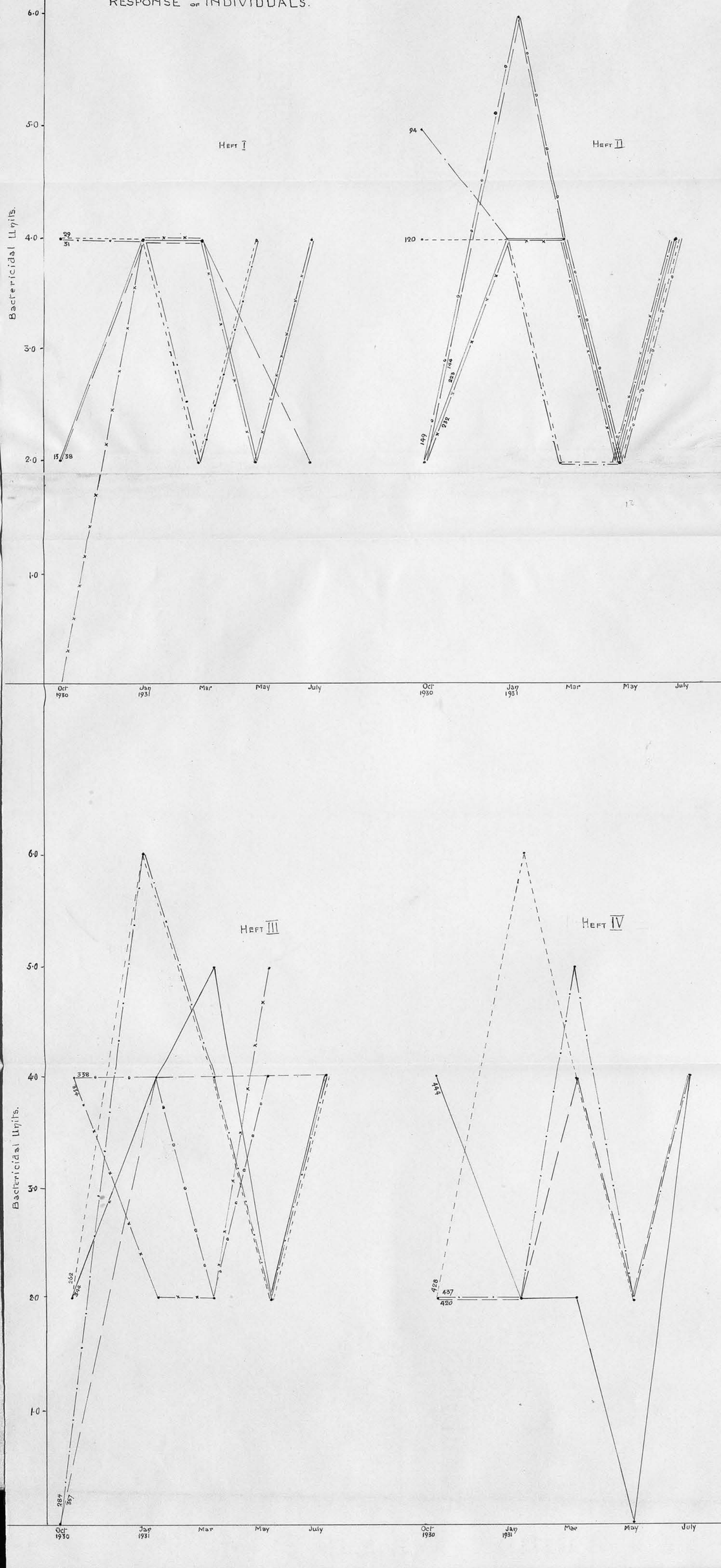
" " Agglutination Reactions are given as agglutination units.

Fig 13.

GARROCHORAN EWES.

BACTERICIDAL REACTION
WITH B. SUIPESTIFER.

RESPONSE OF INDIVIDUALS.



means, with the exception of that date, when the mean of all hefts for the period is given.

The individual figures will be found in the protocols in the appendix and from Table XV.

Bactericidal reaction with B. suipestifer.

Individual variation. In the preliminary test on 7th July, 1930, of 21 sheep tested, 7 showed bactericidal action equal to 2 units and the remaining 14 showed action equal to 4 units, while on the 15th July all 21 ewes gave a response in action of 2 units.

On the subsequent 17 days on which ewes were tested the limits of variation on any day never exceeded 4 units. There was a variation of only one unit on five of these days, of 2 units on six days, of 3 units on five days, and 4 units on the remaining days. On only one of these days did the animals in any one heft show a difference greater than 2 units.

Similar variation occurred among the gimmers and barren ewes, but among the hoggs they were slightly greater. These showed variations of 2 units on two days, of 3 units on three days, of 4 units on two days, and even reached 5 units one day. The limits of variation within any heft were 4 units on 2 days, and 3 units on three days.

Periodic Response of Individuals. The response of individuals to this test at various times have been graphed and figure 13 serves to show the complexity of the material under consideration. In one or two instances the curves for two individuals may run parallel, but this is only when the individuals have always/

always been tested on the same day.

Heft Differences and Differences between Types of Animals: It will be seen from Table XX how very small the differences between the mean values for each heft are compared with the variations between individuals, particularly when the average over any period is considered. This latter occurrence is attributable to the fact that the order of the hefts with regard to strength of reaction does not remain the same on the different days within any period. Often any preced-:ence which a heft can claim one day is cancelled when its mean is lowest of the four on the preceding or subsequent day. For example, on the 19th, 21st, 26th and 28th January, 1930 the order of the hefts as judged by the mean value of the reaction was as follows:-

19th	I, II, III, IV.	
21st	II, III, (I = IV)	The brackets indicate
26th	II, IV, (I = III)	equality of the hefts
28th	(I = II = IV) III.	included.
Period 19th - 28th	II, I, III, IV.	

Again, on the four days in May, 1931, the order was as follows:-

21st	I = II, III, = IV.
23rd	IV, I = II = III.
27th	II = IV, I = III.
29th	III, I = II = IV.
Period 21st - 29th	II, III, = IV, I.

The differences were very small throughout, being negligible in the case of the means over the whole period. Similar inconsistencies appeared in July.

In the three periods cited above, it is impossible to pick out any heft as giving a stronger response to the reaction than the remainder. In March, how-:ever, heft III was highest throughout, though the differences between its means and those of the other hefts were very small. Hefts I, II, and IV did not main-:tain any definite positions relative to one another. In the previous October similar/

Fig 14.

GARROCHORAN HOGGS and GIMMERS.

BACTERICIDAL REACTION

WITH B. SUIPESTIFER.

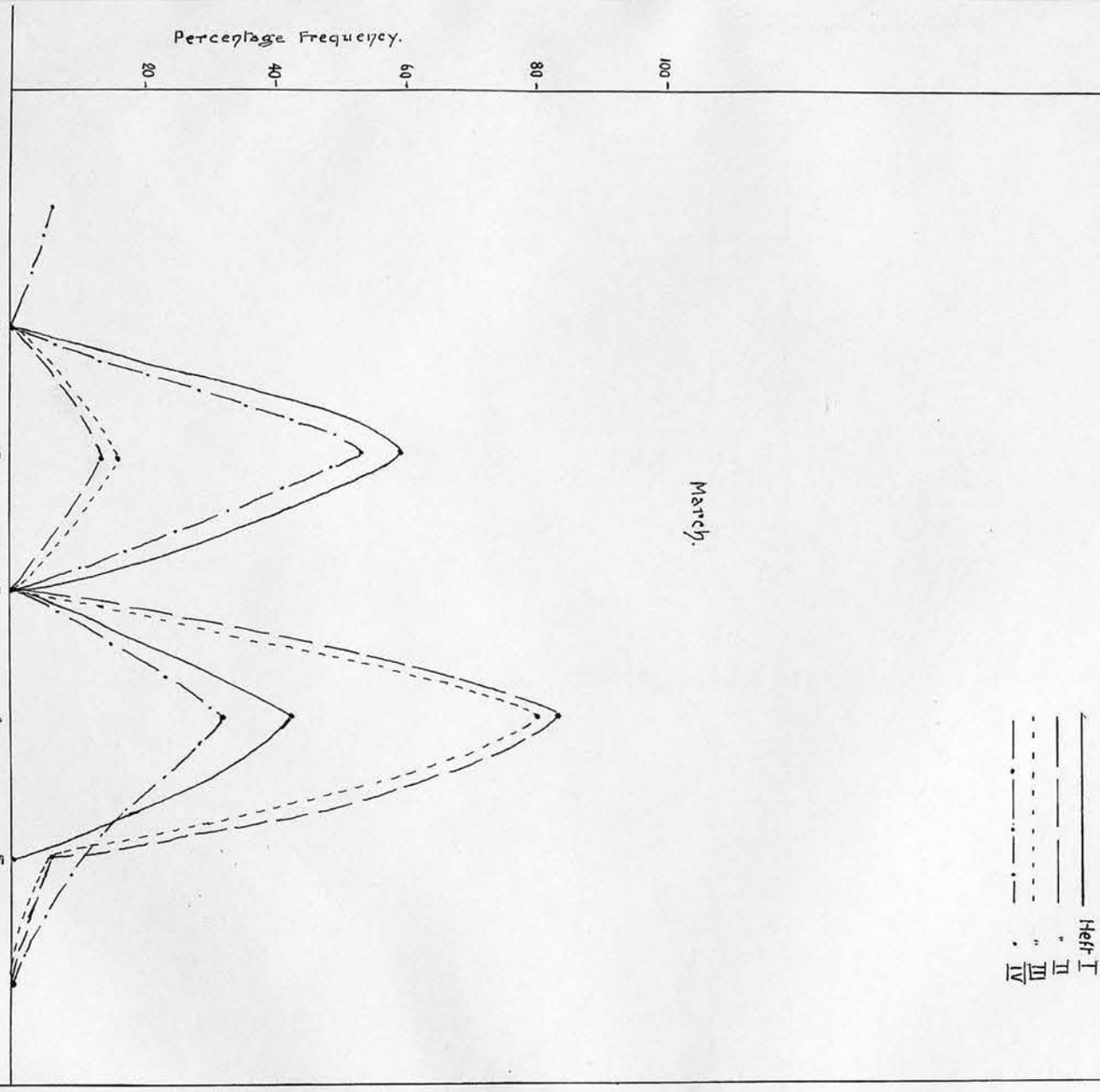
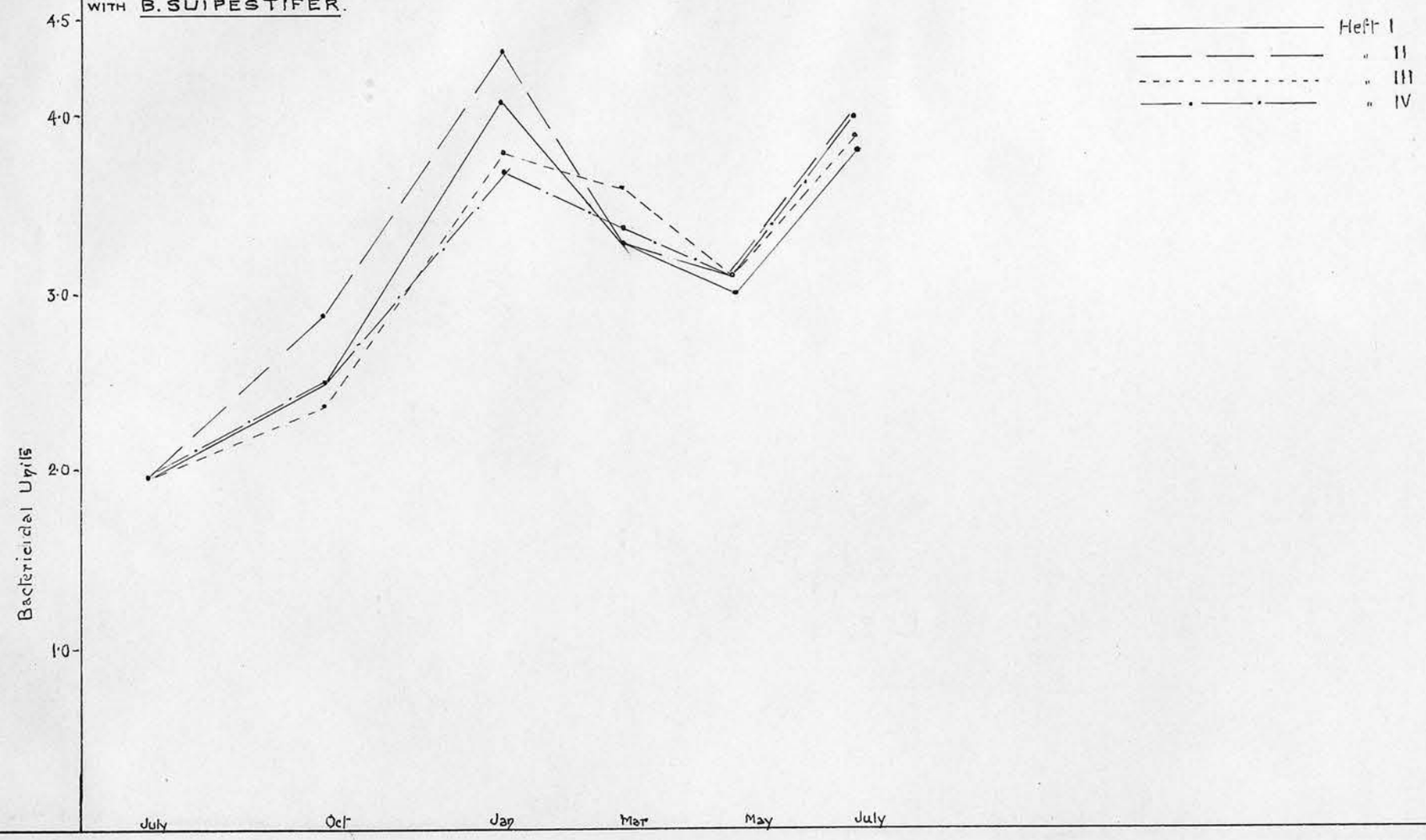


Fig 15.

GARROCHORAN EWES.

BACTERICIDAL REACTION

WITH B. SUIPESTIFER.



similar results were obtained, heft II at this time giving the highest mean throughout, while the others varied.

These observations apply to ewes, but the hoggs show similar inconsistencies. Among them, as among the ewes, heft II was highest in October, but at no other time did any heft behave consistently on the two days of sampling.

It is difficult to say whether any significance can be attached to the single-day observations of the gimmers and barren ewes. In contra-distinction to the results for the ewes and hoggs, heft II was lowest among both gimmers and barren ewes in October, 1930. Heft I was lowest among the gimmers and heft IV among the barren ewes in February.

Frequency curves for the four hefts showing the percentage number of the hoggs and gimmers tested in March which possessed different activity, indicate a very definite parallelism between hefts I and IV, and between hefts II and III (fig. 14). In the latter the peak of the curves is in the region of higher activity than in the former hefts. It must be borne in mind, however, that these hefts were not all sampled each day and that, therefore, daily variation may have played some part in achieving this result.

The ewes in all four hefts behaved similarly from period to period. A rise in the strength of the reaction occurred throughout from July through October to January, most marked in heft II (fig.15). This increase was succeeded by a decrease in strength from January to March and May, occurring most rapidly in hefts I and II and being very slight in hefts III and IV until after March. Finally all hefts rose in July to a level as high as the January readings.

The/

The hoggs behaved in a different manner from the ewes. Hefts I, III and IV increased in reaction from October to January, while heft II decreased slightly. All hefts then increased from January to May. In hefts I and IV the increase was small from January to March and then more marked from March to May. In heft III the greater part of the increase occurred between January and March, but was continued to May. In heft II, however, a large increase occurred from January to March, followed by a small decrease in May. This variation in the behaviour of the hefts in March may be due to all hefts not having been sampled each day, as mentioned above.

Considered heft by heft, the gimmers varied remarkably little in their results in October, February, and June. Hefts I and IV were lower than II and III in April, but this again was probably a daily variation. The barren ewes increased in reaction in hefts I, II, and III from November to February, while heft IV remained stationary. By April this latter heft had reached the same level as the others which showed no change from the February readings.

Comparing the levels of bactericidal activity shown by hoggs' and ewes' sera it will be seen that the means for the October period are higher for ewes than for hoggs. If, however, the means from separate days be examined, it appears that on two of the three days on which ewes were sampled they were very similar to the hoggs sampled on two days, but on the remaining day the ewes were much higher. Thus again it seems that the discrepancy between the period averages at this time is only due to daily variation. On the other hand, the daily means for the ewes in January are all much higher than those for hoggs at this time. In March there is little difference between the two types of animals, the daily variation affecting both similarly.

Ewes/

Ewes were sampled on 21st, 23rd, 27th, and 29th May, and hoggs on 6th and 8th June. The bactericidal activity of the ewes' sera on 21st and 23rd was roughly half of that on the 27th and 29th. The activity of the hoggs' sera showed little difference on the two days and was on a par with that of the ewes on the 27th and 29th. When the mean for the period is considered the ewes showed lower activity than the hoggs.

On account of the daily variation and the fact that barren ewes and gimmers were only tested on one day each in each period, it does not seem permissible to draw any comparison between the results from these animals and from others tested on several days. On the days in April on which hoggs and gimmers or hoggs and barren ewes were sampled together little difference appeared between the different types of animals.

Summary. From consideration of the data collected regarding this bactericidal reaction with B. suipestifer, the most outstanding point that emerges is the consistency of the results from animals sampled on any one day. This consistency appears to be slightly less marked among hoggs than ewes. It has been found difficult to assess differences between the hefts as they so seldom maintained any consistent positions relative to one another. In March, however, heft III gave the highest results on all days, while the relative strength of the other three hefts varied. Any differences between the hefts were very small.

It has not been considered advisable to compare the results obtained from one day's sampling of gimmers with those of two or more days in the case of hoggs and ewes. In October and March there was little difference in the strength/

strength of the reaction given by ewes' or hoggs' sera. In January, on the other hand, the ewes showed greater activity than the hoggs. The daily variation is very noticeable among the ewes in May, the activity of their sera on two days being of the same order as that of the hoggs' sera, while, on the other two days it was roughly half that strength.

Bactericidal Reaction with B. coli "X"
Table XXI.

Individual variation. Until the technique of this test was changed in November, 1931 most of the sera examined showed no bactericidal activity towards B. coli "X" In October, 1930 all results were entirely negative, while in January and March, 1931 there were two and one days respectively on which some individuals showed 2 units of activity. In May and July these higher values became more frequent, appearing on all days. Thus the individual differ-:ences throughout the period from October, 1930 till July, 1931 never exceeded 2 units, which, with the series of centimal dilutions in use, was the smallest observable difference. The refinements and changes in technique in November, 1931 made it possible to estimate one unit of activity. During that month the differences remained within the previous limits, and on two days were even reduced to one unit. On the remaining two days two units of difference were observed. In March, 1932 greater differences appeared, readings of three and four units of bactericidal action occurring together with others of no activity, both over the whole day and in separate hefts. It thus appears that the new technique is more capable of detecting differences than that formerly used.

Periodic Response of Individuals. As in the bactericidal reaction with B. suipestifer the results obtained from individuals at the different samplings did/

TABLE

BACTERICIDAL REACTION

DAILY

Ewes.

	1930 May 27.	Jly. 15.	Oct. 13.	15.	22.	1931. Jan. 19.	21.	26.
Heft I.	-	2.0	1.6	4.0	2.0	4.4	2.8	3.2
II.	-	2.0	2.2	4.2	2.2	3.6	4.0	4.0
III.	-	2.0	1.6	4.2	1.6	3.2	3.2	3.2
IV.	-	2.0	1.6	4.0	2.0	2.4	2.8	3.6
Overall	-	2.0	1.7	4.1	1.9	3.4	3.2	3.5

Gimmers.

	1930 Oct. 27.	1931. Feb. 2.	1931. Apl. 6.	8.	June. 2.
Heft I.	4.5(4)	3.5(4)	-	2.0(4)	4.0
II.	3.6	4.0	4.0	-	4.2
III.	4.4	4.0	4.2	-	4.2
IV.	4.0	4.0	-	2.0(6)	4.0
Overall	4.1	3.9	4.1	2.0	4.1

Month Avge 3.0

PERIOD AVERAGES.

Ewes.

1930 Oct.	1931. Jan.	Mar.	May.	Jly.
2.5	4.1	3.3	3.0	3.8
2.9	4.4	3.3	3.1	4.0
2.4	3.8	3.6	3.1	3.9
2.5	3.7	3.4	3.1	4.0
2.6	4.0	3.4	3.1	3.9

Hoggs.

1930 Oct.& Nov.	1931. Feb.	Apl.	June.
1.8	2.3(9)	2.6	4.1
2.2	2.0(9)	3.9(8)	4.0
2.0	2.2	3.4	4.0
1.6	2.5(9)	2.9(8)	4.1(9)
1.9	2.2	3.2	4.0

WITH B. SUIPESTIFER.

AVERAGES. (in bactericidal units)

1931 Jan. 28.	Mar. 23.	25.	30.	Apl. 1.	May. 21.	23.	27.	29.	Jly. 20.	22.
6.0	4.0	2.0	-	3.8	2.0	2.0	4.0	4.2	3.6	4.0
6.0	4.0	2.2	3.8	-	2.0	2.0	4.2	4.2	4.0	4.0
5.6	4.6	2.2	4.1	-	1.6	2.0	4.0	5.0	4.2	3.6
6.0	4.4	2.0	-	3.8	1.6	2.2	4.2	4.2	4.0	4.0
5.9	4.2	2.1	3.9	3.8	1.8	2.1	4.1	4.4	3.9	3.9

Hoggs.

1930 Oct. 29.	Nov. 6.	1931. Feb. 4.	11.	Apl. 6.	8.	13.	15.	June. 4.	8.
1.6	2.0	1.8	3.0(4)	-	2.0	-	3.2	4.4	3.8
2.0	2.4	1.6	2.5(4)	3.8	-	4.0(3)	-	4.0	4.0
2.0	2.0	1.6	2.8	3.6	-	-	3.2	4.8	3.2
2.0	1.2	2.2(4)	2.8	-	1.5(4)	4.2(4)	-	4.2	4.0(4)
1.9	1.9	1.7	2.8	3.7	1.8	4.1	3.2	4.3	3.7

Barren Ewes.

1930.	1931.		
Nov.	Feb.	Apl.	
3.	9.	13.	15.
2.6	3.6	-	4.0
2.0	4.0	3.6	-
2.5(4)	4.0	-	4.0
2.8	2.8	3.8	-
2.5	3.8	3.7	4.0
		Month Avge.	
		3.8	

did not show any parallelism. Similarity in behaviour was only found in animals sampled together at the various periods.

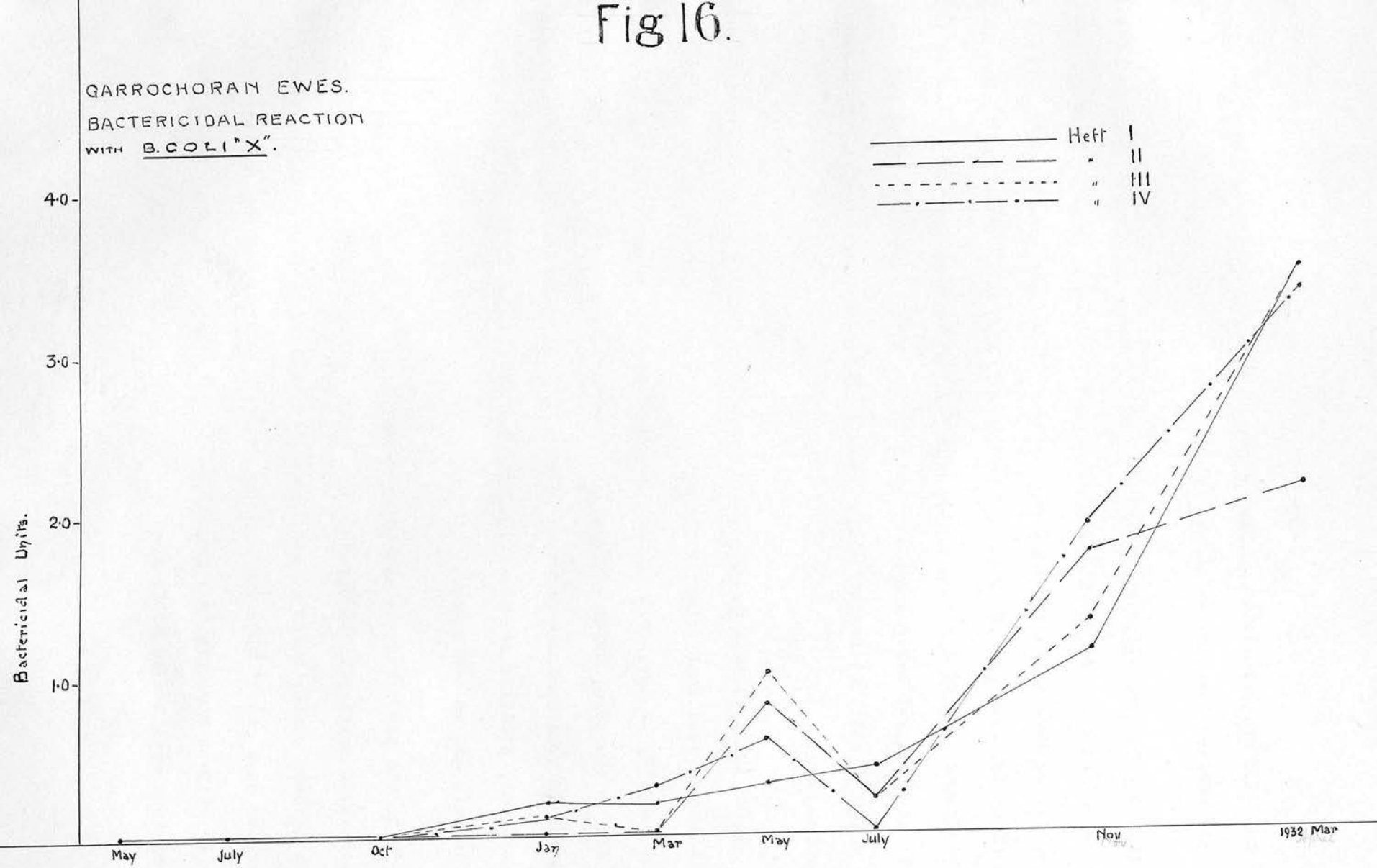
Differences between Hefts and between Different Types of Animals - Ewes. Until the end of March, 1931 samplings no heft differences can be said to have appeared, as almost all readings were negative. On 21st January, 1931, heft I showed on the average 0.8 units of bactericidal activity, and heft IV, 0.4 units, while on the 28th of the same month, heft III gave a mean reading of 0.4 units. Again on April 1st hefts I and IV (the only two hefts sampled) showed 0.4 and 0.6 units of activity respectively. After that date positive readings became more frequent.

As in the test with B. suipestifer already discussed the hefts failed to maintain any positions relative to one another from day to day. Of the four days' sampling in May, heft II gave the highest reaction on the 21st, hefts II and III on the 23rd, hefts III and IV on the 27th, and heft III on the 29th. Heft III is thus dominant for that period. In July when two days sampling was carried out, positive reactions occurred in hefts I and II on the first day and in heft III on the second.

With the new technique adopted in November, 1931 the same irregularities in behaviour appeared. On three of the eight days on which samples were taken in that month, hefts II and IV occupied the two highest places, and on other two days one of them was highest. The means for the whole period place heft IV highest and heft II second. In March, 1932 the order of the hefts on the various days was as follows:-

Fig 16.

GARROCHORAN EWES.
BACTERICIDAL REACTION
WITH B. COLI "X".



March 21st	I, > IV, >II, >III.
" 23rd	I, >II, = III, = IV.
" 28th	III, >I, >II, >IV.
" 30th	III, > II = IV, >I.
April 11th	III, >IV, >I, >II.
" 13th	IV, >I, >III, >II.
Complete period.	III = I, > IV, >II.

Heft II throughout gave indications of lower activity than the other hefts, which all varied considerably.

Thus among the ewes, the only remarkable points are that heft III was highest in May, 1931, that hefts II and IV appear to be higher than I and III in November, 1931 and that heft II was low in the following March.

Hoggs. No consistent differences could be discerned among these results.

Gimmers. On 2nd February, 1931 heft II was higher than the other hefts, but no further differences occurred.

Barren Ewes. As with the gimmers, heft II was the highest at the February sampling, but no other differences appeared.

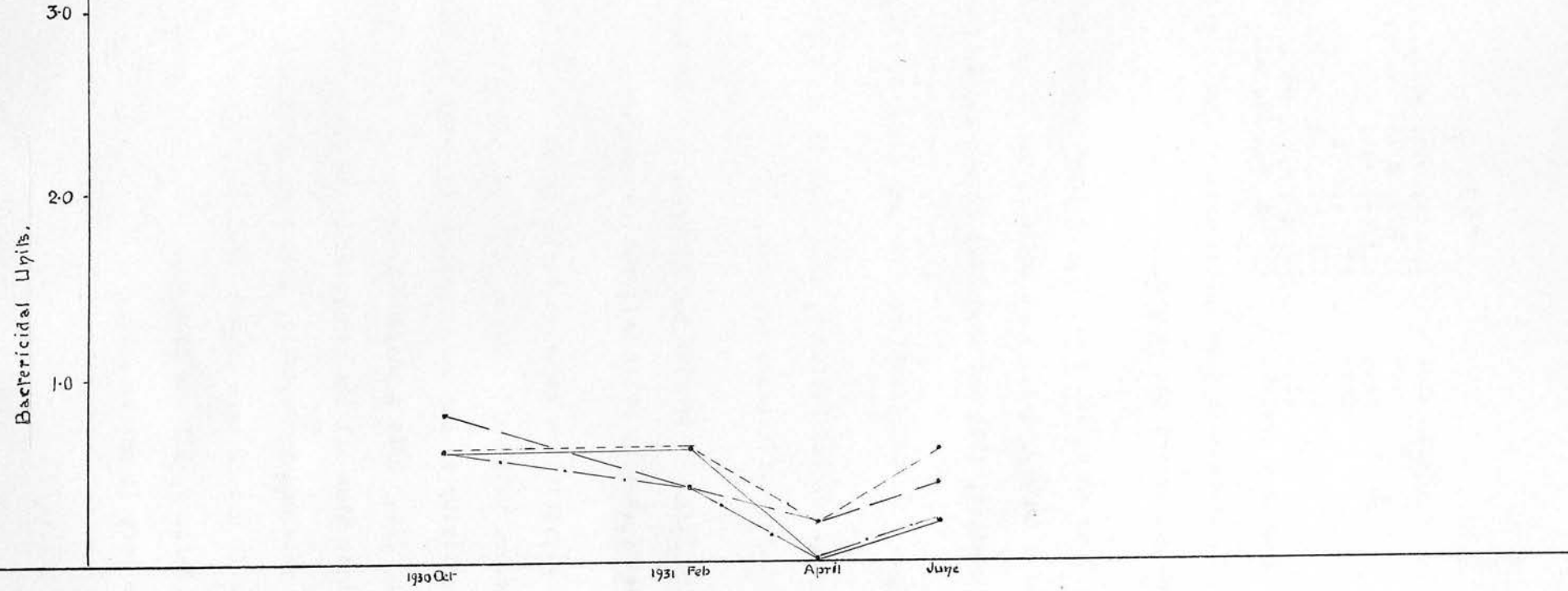
No variation occurred in the behaviour of the hefts (ewes) from period to period until after March, 1931 (fig.16). From March to May an increase in the activity of the sera appeared in hefts II, III and IV, most marked in hefts II and III. Heft I stayed at almost the March level in May but rose in July, by which time all the other hefts had fallen considerably. Much higher results were obtained in November, 1931 than previously, due to the new technique. Hefts IV and II were then highest with III and I 0.6 units below them. By March 1932 hefts I, III and IV showed a marked increase to practically the same value, while heft II was considerably lower.

Little/

Fig 17.

GARROCHORAN HOGGS.
BACTERICIDAL REACTION
WITH B. COLI "X".

Heft I.
" II.
" III.
" IV.



Little difference occurred among the hoggs from period to period (fig.17). Heft II was highest in October, but fell away, along with heft IV in January, while hefts I and III maintained their October level. All fell in March, hefts II and III remaining slightly higher than I and IV. By May they had risen again somewhat and the order was II > III > I = IV.

Both gimmers and barren ewes gave a higher figure in February than at any other time and heft II rose to the highest level in both types of animals.

In October while ewes, barren ewes and gimmers gave entirely negative results in this test, the hoggs showed activity in all hefts. In the January-February period, however, though remaining higher than the figures for the ewes, the results obtained from the hoggs were not so high as those from gimmers or barren ewes. In March and April all types of animals were similar, but in May and June the ewes gave higher results than the hoggs, while the gimmers displayed no bactericidal action.

Summary. Until the May sampling very few sera showed any bactericidal action to B. coli "X". After that date more positive reactions occurred, but until the technique was changed in November, no activity higher than 2 units was observed. Later, three and four units of activity were noted.

Among the ewes, heft III was highest in May, 1931, and hefts II and IV were higher than I and III in November, though not consistently. In March, 1932 heft II was noticeably low. The hoggs showed no consistent differences at any time, nor did the barren ewes or gimmers except in February 1931, when heft II was highest.

While/

While ewes gave consistently negative results until May, among the hoggs there were some positive reactors at all samplings. The figures for gimmers and barren ewes in February are so much higher than for the ewes sampled at this time as to appear outwith the limits of daily variation. The results for the hoggs are intermediate between those for ewes on the one hand, and for barren ewes and gimmers on the other. At other times no differences appeared among the different types of animals.

Bactericidal Reaction with Streptococcus haemolyticus.

When this reaction was first included among those in use, the series of dilutions of bacterial emulsion was a centimal one. This meant that the smallest difference in bactericidal action that could be observed was one of two units. In November, 1931 the series was changed to include some decimal dilutions, and smaller differences could then be seen.

During January, 1931, (the first time this test was used), few of the ewes' sera showed any bactericidal action, those which did so not exceeding two units. At the following sampling the majority of the results were again negative, though two and four units of activity were recorded from some individuals. In May and July two units were again observed, more frequently in the latter month.

After the introduction of the decimal dilutions in November, observations of no activity became less frequent, and some sera displayed five units of action. Readings which differed from each other by five units were made on the same day, while four units was the greatest difference observed between readings in one heft. Such variations seldom appeared, however, the majority of the results from any heft on a given day not exceeding two units, and from

all/

TABLE

BACTERICIDAL REACTION

WITH B. COLI "X"

DAILY

AVERAGES. (in bactericidal units)

Ewes.

	1930. May 27.	Jly. 15.	Oct. 13.	15.	22.	1931. Jan. 19.	21.	26.
Heft I.	0	0	0	0	0	0	0.8	0
II.	0	0	0	0	0	0	0(2)	0
III.	0	0	0	0	0	0	0	0
IV.	0	0	0	0	0	0	0.4	0
Overall	0	0	0	0	0	0	0.3	0

	Mar. 28.	23.	25.	30	Apl. 1.	May. 21.	23.	27.	29.	Jly. 20.
Heft I.	0	0	0	-	0.4	0	1.2	0.4	0.8	0.8
II.	0	0	0	0	-	0.4	1.6	0.8	0.4	0.4
III.	0.4	0	0	0	-	0	1.6	1.2	1.2	0
IV.	0	0	0	-	0.6	0	1.2	1.2	0	0
Overall	0.1	0	0	0	0.5	0.1	1.4	0.9	0.6	0.3

Gimmers.

	1930. Oct. 27.	1931. Feb. 2.	Apl. 6.	8	June. 2.
Heft I.	0(4)	1.0(4)	-	0(4)	0
II.	0	1.6	0.4	-	0
III.	0	0.4	0.4	-	0
IV.	0	0.8	-	0	0
Overall	0	0.9	0.2	0	0

Month Avge 0.1

Hoggs.

	1930. Oct. 29.	Nov. 6.	1931. Feb. 4.	11.	Apl. 6.	8.	13.	15.	June. 4.	8.
	0.8	0.4	0	1.2	-	0	-	0	0	0.4
	0.4	1.2	0	0.8	0	-	0.4	-	0.4	0.4
	0.4	0.8	0	1.2	0.4	-	-	0	0.8	0.4
	1.2	0	0(4)	0.8	-	0	0	-	0	0.5
	0.7	0.6	0	1.0	0.2	0	0.2	0	0.3	0.4

PERIOD AVERAGES.

Ewes.

	1930. Oct.	1931. Jan.	Mar.	May.	Jly.	Nov.	1932. Mar.
Heft I.	0	0.2	0.2	0.3	0.4	1.1(38)	3.5
II.	0	0 (17)	0	0.8	0.2	1.7	2.1
III.	0	0.1	0	1.0	0.2	1.3	3.5
IV.	0	0.1	0.3	0.6	0	1.9	3.4(29)
Overall	0	0.1	0.1	0.7	0.2	1.5	3.1
	(15)	(20)	(20)	(20)	(10)	(40)	(30)

(The figures in brackets below the period averages indicate the number of results from which those averages are calculated).

Hoggs.

1930. Oct.	1931. Feb.	Apl.	June.
0.6	0.6	0	0.2
0.8	0.4	0.2	0.4
0.6	0.6	0.2	0.6
0.6	0.4(9)	0	0.2
0.6	0.5	0.1	0.3
(10)	(10)	(10)	(10)

1930. Nov. 22.	Nov. 2.	5.	9.	11.	17.	18.	23.	25.	1932. Mar. 21.	23.	28.	30.	Apl. 11.	13.
0	0.2	0.4	0	0.6	1.0(2)	0.6	1.0	1.0	2.6	2.0	1.0	1.0	2.8	1.0
0	0.6	0.4	0	0.8	1.6	0.4	1.0	2.0	2.2	1.0	0.8	1.2	0.8	0.4
0.4	0.4	0.4	0.4	0.6	0.6	0.8	0.8	1.4	1.6	1.0	1.8	2.2	3.2	0.8
0	0.4	1.4	0	1.0	1.2	0.8	0.6	2.2	2.4	1.0	0(1)	1.2	3.0	1.2
0.1	0.4	0.6	0.1	0.7	1.1	0.6	0.8	1.6	2.2	1.2	1.1	1.4	2.4	0.8

Barren Ewes.

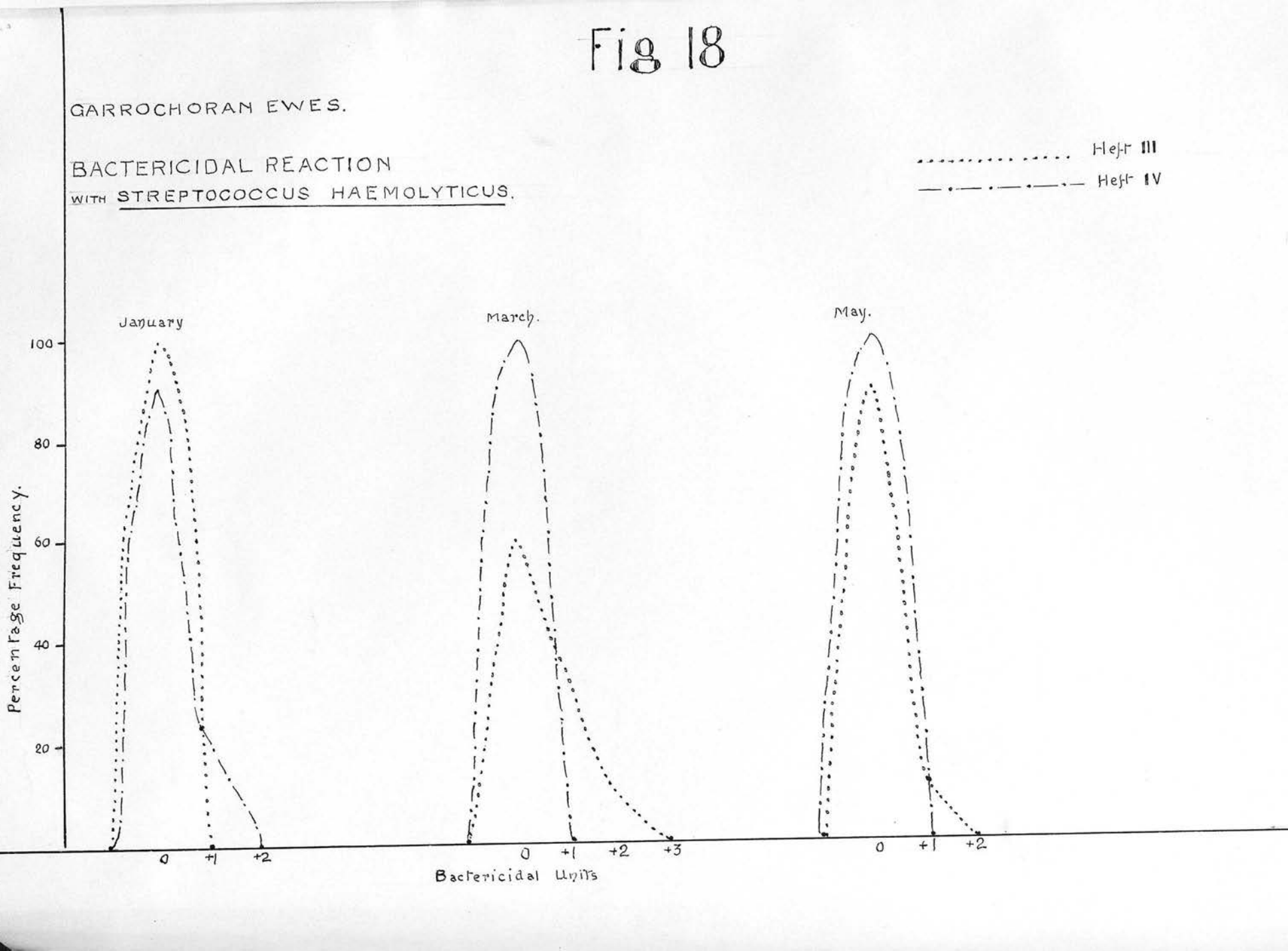
1930. May. 7.	Jly. 7.	Nov. 3.	1931. Feb. 9.	Apl. 13.	15.
0.4	0	0	0.4	-	0
0	0	0	1.2	0.8	-
0.6	0	0	0.8	-	0
0	0	0	0	0	-
0.2	0	0	0.6	0.5	0

Month Avge
0.2

Fig 18

GARROCHORAN EWES.

BACTERICIDAL REACTION
WITH STREPTOCOCCUS HAEMOLYTICUS.



all hefts on one day not exceeding three units.

It was noticed in the bactericidal reaction with B. coli "X" that the variations among the hoggs were greater than among the ewes. This is also seen in the present reaction. On 8th April, 1931 eight and six units of bactericidal action were recorded (one case of each), while other sera on the same day showed no activity. At this time four units of activity were more frequently observed among all other types of animals than among ewes. In January and May the differences were of the same order as among the ewes.

Periodic Response of Individuals. The variation in the response of individuals from time to time was found to be considerable, as was the case in the two reactions previously discussed.

Difference between Hefts and between Types of Animals - Ewes. As will be seen from Table XXII, the results in January were almost entirely negative, though positive bactericidal action was shown by heft III on two days and by heft I on a third day. In March heft III was highest of all hefts on one day and higher than heft II on another. (Only the two hefts were sampled on the latter day). In May this test was only carried out on two days, and while no activity whatever appeared on one of these, in the other hefts I and III showed positive results, heft I to the greater extent. No real difference appeared in July. Frequency distribution curves for hefts III and IV show how the higher figure for heft III in March was due to the occurrence of more positive reactions in that heft (fig.18).

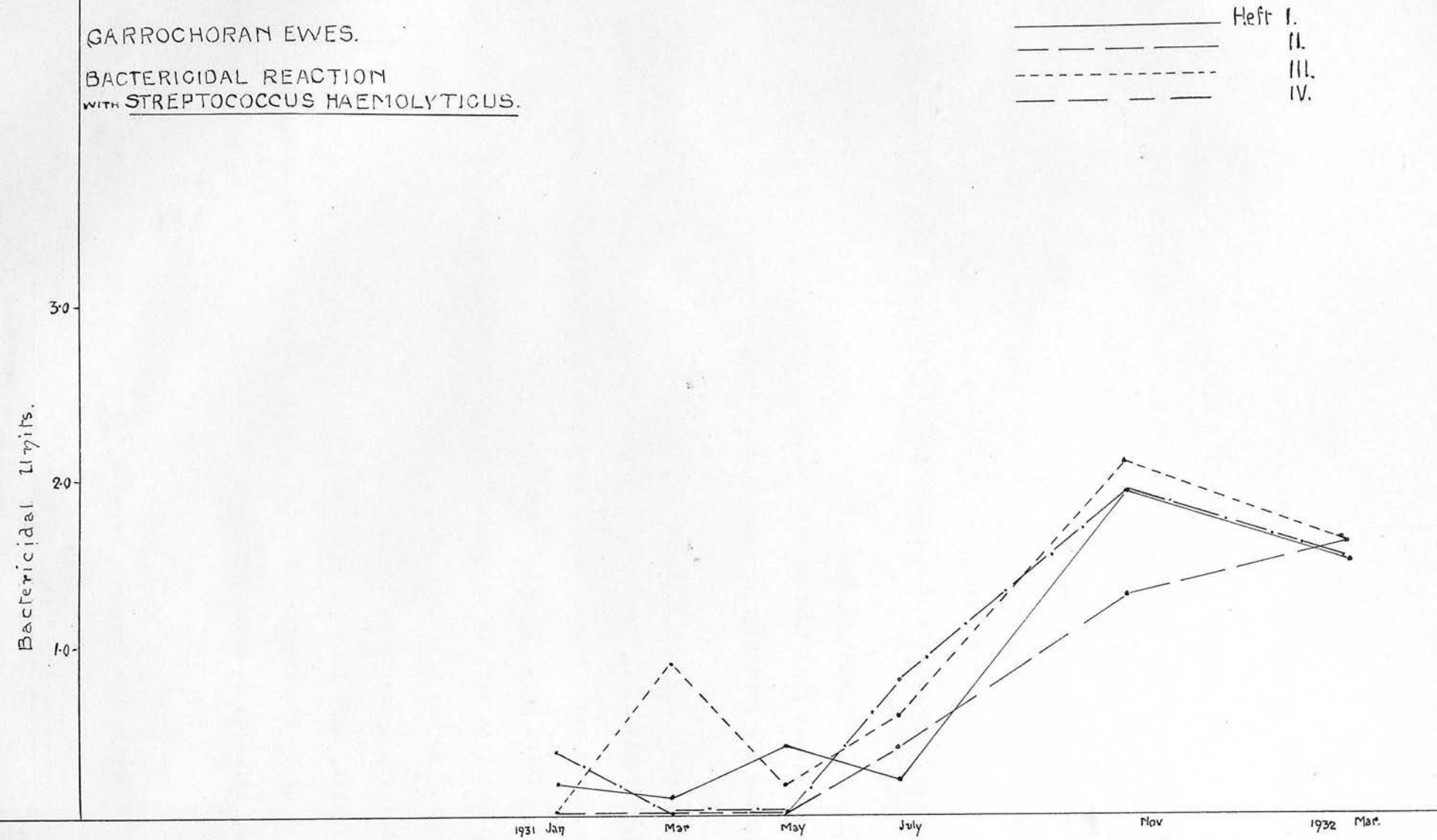
During the November samplings the order of the hefts was as follows:-

November /

Fig 19.

GARROCHORAN EWES.

BACTERICIDAL REACTION
WITH STREPTOCOCCUS HAEMOLYTICUS.



November 5th	I > III = IV > II.
" 19th	III > I > IV > II
" 11th	I > III > II > IV.
" 17th	III = IV > I > II.
" 18th	I > IV > II > III.
" 23rd	III > I > IV > II.
" 25th	III > IV > II > I.
Period 5th - 25th.	III > I = IV > II.

Heft II throughout occupies a consistently low position while I and III are usually fairly high. The averages over the whole period place III highest. In March, 1931 no consistency appears in the relative positions of the hefts and the averages of the different hefts for the period only differ by 0.1 units.

Hoggs, Barren Ewes and Gimmers. On the one day on which this reaction was tested in June, 1931 the order of the hefts was III, I, IV, II, the results for heft III and I being twice as great as for II and IV. No other marked differences appear among the hoggs or gimmers. Heft II of the barren ewes in February, 1931 gave much higher figures than any of the other hefts.

The period averages for the ewes of heft I showed little difference from January till July, 1931, then rose considerably in November and March, 1932 (fig.19). Hefts II and IV behaved similarly, the rise in November being somewhat less in heft II. Heft III showed a considerable rise in March, 1931, then fell again to the level of the other hefts in May and July. In November it was slightly higher than the others but was the same again in March, 1932.

Heft I of the hoggs rose very considerably from February to April then fell again by June. Hefts II and IV behaved similarly but the April rise was not nearly so marked. Heft III showed a certain rise in April which

continued/

continued to a slight extent in June.

The gimmers and barren ewes in all the hefts exhibited no real variation in behaviour from period to period.

When the ewes and hoggs are compared no difference appears between them in the January-February period. In March and April the general average is much higher for the hoggs than the ewes and in May and June this difference again appears but to a less marked extent.

The average figures for ewes, barren ewes and gimmers are practically the same for the January-February period. In March-April the similarity continues between the ewes and barren ewes, but the figure for the gimmers approximates more nearly to that for the hoggs at this date. In May-June it has returned again to the value of the ewes.

Summary. While the individual variation among the ewes was only two units, except on one day, during the first four periods that this reaction was examined, it was greater among the other types of animals, particularly hoggs, in March, 1931. In November, 1931 and March, 1932 greater variations were observed and results differing by as much as five units among all animals or by four units among animals in one heft were noted on a single day. The usual variation was, however, three units in the one case and two in the other.

Among the ewes no heft differences occurred in January but in March there were indications that heft III was highest. This was complicated by the separation of the hefts on the last two days of that period. May and July results showed no differences. In November, 1931 heft II maintained a low

position/

position throughout, while hefts I and III shared the highest place. The order of the hefts as determined by the averages for the period is III, (I and IV), II. In March, 1932 no consistent heft differences appeared. The only remark-:able variations among the other types of animals were in June, when hefts I and III of the hoggs gave results twice as great as those of hefts II and IV, and in February when heft II of the barren ewes was definitely highest of the hefts. While hefts I, II and IV of the ewes showed no difference from January to July, 1931, then rose in November and March, heft III rose consider-:ably above the level of the others in March, 1931, but returned to the level of the other hefts at the remaining periods. At the March-April sampling, and to a less extent at the following one in May, the hoggs gave much higher results than the ewes.

Haemolytic Reaction with Rabbit Erythrocytes.

Individual Variation. During the preliminary tests in May and July, 1930 the individual variation observed amounted to 0.5 and 1.5 M.H.D.s per c.c. respectively, among the ewes. The greatest variation within any one heft was 0.4 M.H.D.s per c.c. in May and 1.5 M.H.D.s per c.c. in July.

In the following October the greatest variation between individual results on any one day was 1.8 M.H.D.s per c.c., and this also was recorded within one heft. The results in another heft on the same day differed only by 0.3 M.H.D.s per c.c. The following January the limits of the greatest individual variation on one day were 2.0 M.H.D.s per c.c. apart and within any heft they were 1.5 M.H.D.s per c.c. apart. In March these figures became 1.5 and 1.3 M.H.D.s per c.c. respectively and in May they reached 3.2 and 3.0 M.H.D.s. These latter were the greatest variations recorded, as in the

following/

TABLE

BACTERICIDAL REACTION

DAILY

Ewes.

	1931. Jan. 19.	21.	26.	28.	Mar. 23.	25.	30.	Apl. 1.
Heft I.	0	0.8	0	0	-	0.4	-	0(10)
II.	0	0	0(2)	0	-	0	0.4(10)	-
III.	0(2)	0	0	0	0(1)	0.8	1.1(9)	-
IV.	0.4	0(4)	0	1.2	-	0	-	0(8)
Overall	0.1	0.2	0	0.3	0	0.3	0.5	0

Gimmers.

	1931. Feb. 2.	Apl. 6.	8.	June. 2.
Heft I.	0(4)	-	0(4)	0.6(3)
II.	0	0.4	-	0(2)
III.	0.4	0.6(3)	-	0(3)
IV.	0.4	-	2.3(6)	0.4
Overall	0.2	0.5	1.4	0.3

Month Avge 1.0

PERIOD AVERAGES.

Ewes.

	1931. Jan.	Mar.	May.	July.	Nov.	1932. Mar.
Heft I.	0.2	0.1	0.4	0.2	1.9(31)	1.5(26)
II.	0(17)	0	0	0.4	1.3(26)	1.6(26)
III.	0(17)	0.9	0.2(9)	0.6	2.1(24)	1.6(29)
IV.	0.4(19)	0(13)	0	0.8	1.9(25)	1.5(23)
Overall	0.2 (20)	0.3 (15)	0.2 (10)	0.5 (10)	1.8 (35)	1.5 (30)

(The figures in brackets below the period averages indicate the number of results from which those averages are calculated),

XXII.

WITH STR. HAEMOLYTICUS.

AVERAGES. (in bactericidal units)

	May. 21.	23.	27.	29.	Jly. 20.	22.	Nov. 2.	5.	9.	11.
	-	0.8	-	0	0.4	0	-	2.4	2.0(4)	2.7(3)
	-	0	-	0	0.8	0	-	1.6(3)	1.6(3)	1.6
	-	0.4(4)	-	0	0.8	0.4	-	2.0(2)	2.3(3)	2.2
	-	0	-	0	0.8	0.8	-	2.0(2)	2.0(1)	1.3(3)
	-	0.3	-	0	0.7	0.3	-	2.1	2.0	2.0

Hoggs.

	1931. Feb. 4.	11.	Apl. 6.	8.	13.	15.	June. 4.	8.
	-	0.4	-	4.4	-	1.6	-	0.8
	-	0	2.0	-	0.4	-	-	0.4
	-	0	0.4	-	-	1.0	-	1.0
	0(4)	0	-	1.0(4)	0.4	-	-	0.5(4)
	0	0.1	1.0	3.0	0.4	1.3	-	0.7

Hoggs.

1931. Feb.	Apl.	June.
0.4(5)	3.0	0.8(5)
0(5)	1.0	0.4(5)
0(5)	0.7	1.0(5)
0(9)	0.7(9)	0.5(4)
0.1 (10)	1.4 (10)	0.7 (5)

				1932. Mar.				Apr.	
17.	18.	23.	25.	21.	23.	28.	30.	11.	13.
0.8	3.2(4)	3.0(4)	0.3(6)	3.2	0.4	2.2(4)	1.0(3)	1.0	0.7(4)
0.7(4)	2.2	2.0(1)	0.4	2.8	0.8	2.0(4)	2.0(3)	1.2	1.0(4)
1.0(3)	2.0(3)	3.6(3)	1.6	1.8	1.6	2.8	0.6	1.2	0(1)
1.0(4)	2.6	2.8	1.2	2.8	0.8	2.4	0.1	1.2	0(3)
0.9	2.5	3.0	0.9	2.6	0.9	2.4	1.0	1.1	0.6

Barren Ewes.

1931. Feb.	Apl.	
9.	13.	15.
0	-	1.2
1.2	0	-
0.4	-	0.4
0	0.4	-
0.4	0.2	0.8

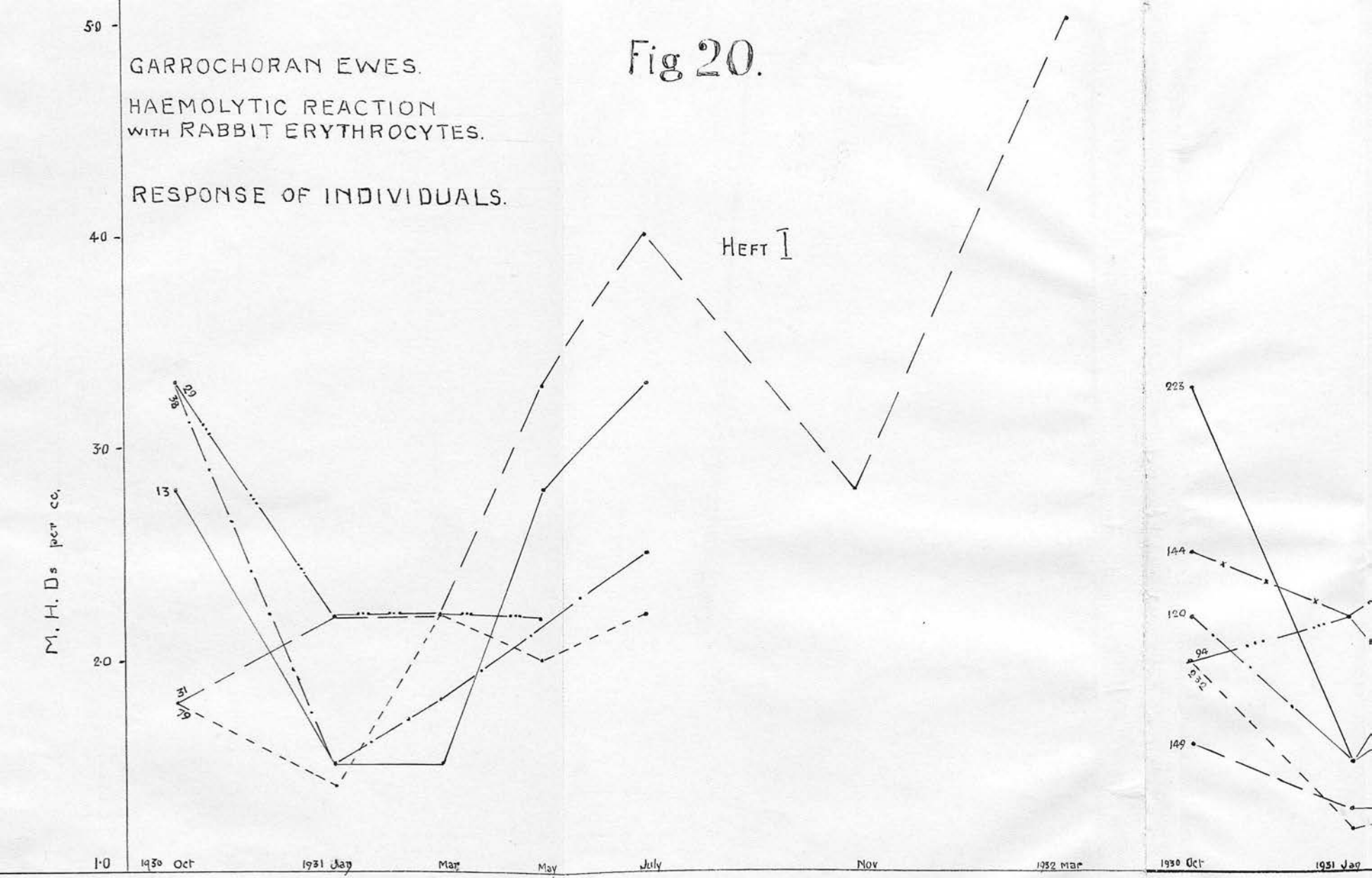
Month Avge
0.5

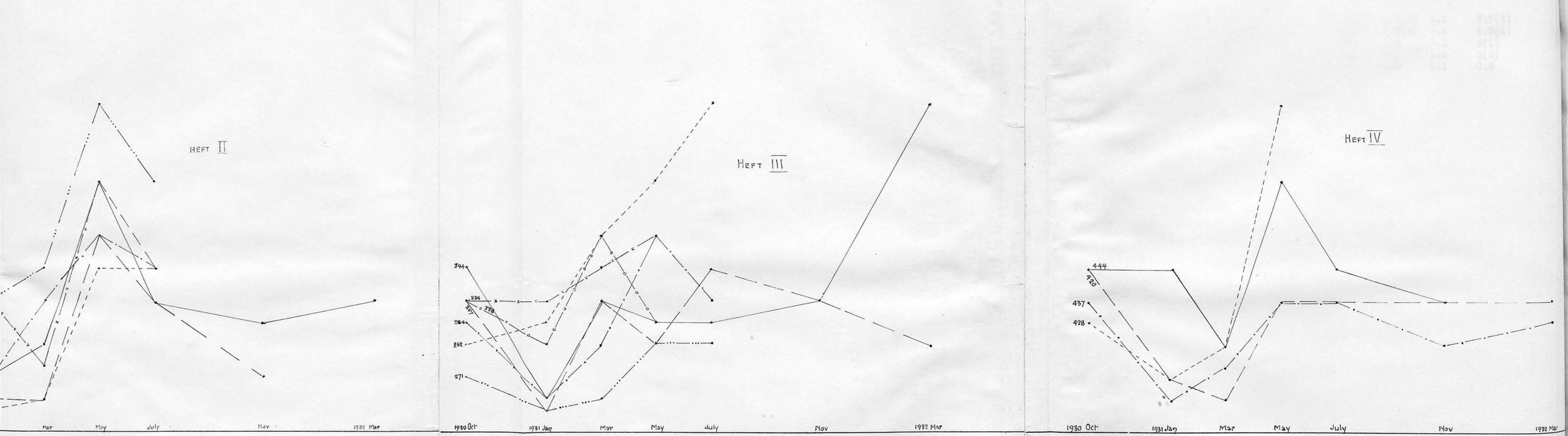
Fig 20.

GARROCHORAN EWES.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES.

RESPONSE OF INDIVIDUALS.




HEFT II
HEFT III
HEFT IV
344
334
339
284
262
271
444
420
437
428
Mar
May
July
Nov
1932 Mar
1930 Oct
1931 Jan
Mar
May
July
Nov
1932 Mar
1930 Oct
1931 Jan
Mar
May
July
Nov
1932 Mar

following July, November and March the above figures became 3.0 and 2.0; 2.8 and 2.8; 3.2 and 3.2 M.H.D.s per c.c.

Thus the greatest individual variation observed throughout was 3.2 M.H.D.s per c.c. for animals sampled on one day and the same variation occurred between animals in one heft on one day. Similar variations were observed among the other types of sheep.

Periodic Response of Individuals. Figure 20 shows how individual animals differ entirely in their response to this test at the various periods of sampling. It is difficult to pick out any two curves which behave similarly, in any of the hefts. In heft III the curves for Nos. 344 and 357 run roughly parallel, as do those for 334 and 262, but there is no similarity whatever between the two pairs of curves.

Differences between Hefts and between Types of Animals - Ewes. (Table XXIII) The differences between the hefts in May and July, 1930 were very small, heft III being highest in May and heft II in July. On the first two days of the following October the order of the hefts was I > II = III >IV. On the third day, however, it was entirely different, II = IV >III >I. Thus heft I gave the strongest reactions on two days; on the third it gave the weakest. The averages for the hefts in this period are all very similar. In January the hefts did not maintain any consistent positions relative to one another on the various days of sampling. In March heft III gave the highest results on all days, while heft IV gave the lowest. In May the order of the hefts varied as shown below:-

May	21st	I	II	IV	III.
"	23rd	I	II	IV	III.
"	27th	IV	I	II	III.
"	29th	I	III	IV	II.
Whole period.		I	IV	II	III.

Heft I remained at the higher end of the scale throughout this series of samplings and heft III was lowest on three days out of four. In July when sampling was carried out on two days, the order of the hefts was exactly opposite on the first day from that on the second. The highest position in November, 1931 was occupied by heft II on four days out of seven, while on another day it was second highest. Heft IV was at the lower end of the scale on six days out of the seven, and on the seventh it was highest. In March, 1932 no consistent behaviour was observed.

Hoggs. No differences appeared between the hefts in the October-November samplings nor on the first day of the February tests. On the second day of this latter period, however, heft I was markedly above the level of the other hefts, of which heft II was lowest. As usual the April results are complicated by the sampling of different hefts on different days. In June no heft can be selected as maintaining any position, high or low.

Gimmers. The mean result for heft II in February was higher than those for the other hefts, and in April heft III appeared to be highest, while in June heft I gave the greatest number of M.H.D.s per c.c.

Barren Ewes. Heft IV was highest in the preliminary tests in May, 1930, and heft III in the following July. In November of that year heft I was lower than the other hefts, which were very similar to one another, while in February and April it was highest.

Figure/

Fig 21.

GARROCHORAN EWES.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES.

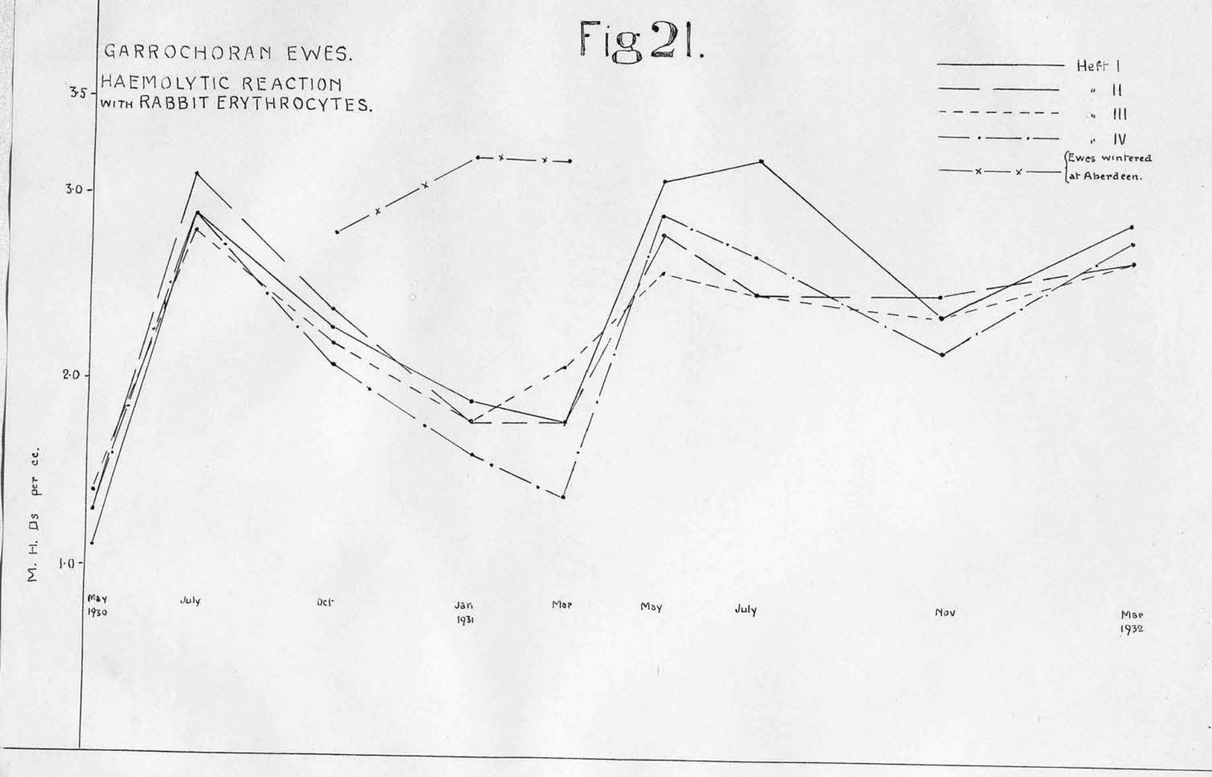


Fig 22.

GARROCHORAN EWES.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES.

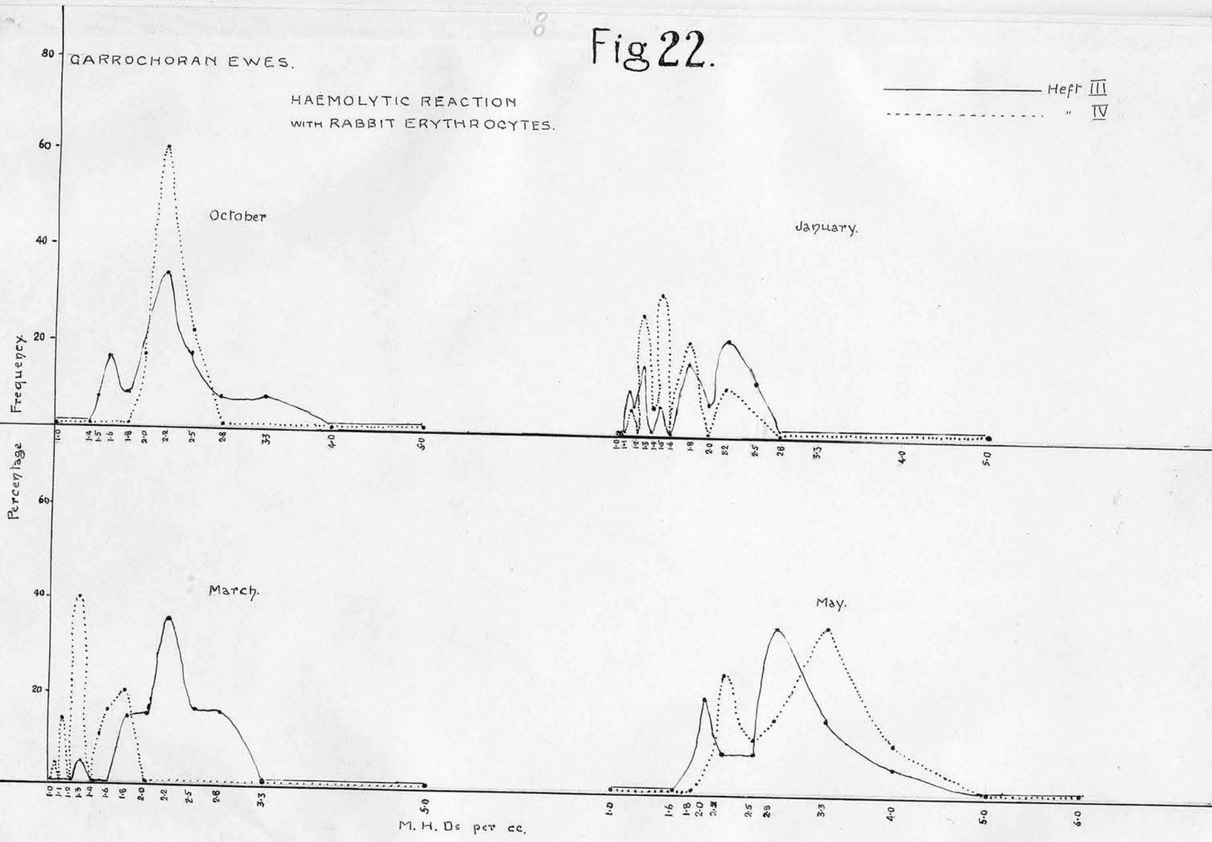


Fig 23.

GARROCHORAN HOGGS.

HAEMOLYTIC REACTION
WITH RABBIT ERYTHROCYTES.

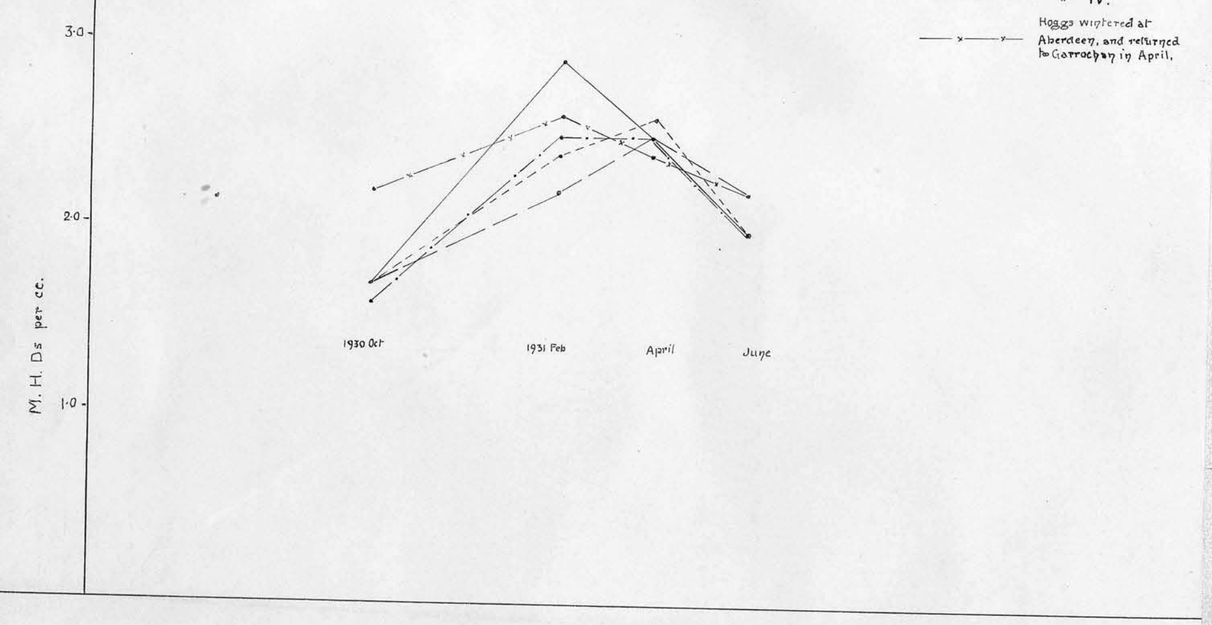


Figure 21 shows the behaviour of the different hefts of ewes from period to period. The curve for each heft rises from May, 1930 to July, and falls through October to January, 1931. Thereafter a difference may be observed between the behaviour of the curves for hefts I and IV, and those for hefts II and III. Hefts I and IV continue to fall from January to March and then rise markedly in May, and after remaining almost constant till July, fall to the November reading and rise again in March, 1932. (It is noteworthy that this recovery occurred earlier in 1932 than in 1931). Hefts II and III, on the other hand, do not fall further after January, 1931. Heft II remains at the same level in March, shows a marked rise in May to a level from which it hardly varies till March, 1932. Heft III behaves similarly, except that the rise commences in March, 1931. Thus while heft IV was lowest in the winter months, it rose to the level of the other hefts during the summer samplings. Figure 22 shows by frequency distribution curves the relationship between hefts III and IV at the various samplings from October, 1930 to July, 1931.

Similar behaviour occurs among the hoggs to a certain extent (figure 23). In this case a rise occurs in all hefts from October to January, and is maintained till March in hefts II and III, while heft I drops in value and heft IV remains stationery. All hefts then fall in value from March to May.

The usual complications arise when dealing with the barren ewes and gimmers, namely, that samples were taken on one day only in each period.

It will be seen that the behaviour of the ewes is entirely different to that of the hoggs throughout the various periods from October, 1930 to June, 1931. At the October-November sampling the hoggs gave lower results than the ewes/

ewes. Then, while the general reaction level of the ewes fell in January and was still low in March, but rose again in May, that of the hoggs exhibited exactly the opposite type of behaviour, i.e. it rose in January, remained steady in March and fell again in May.

The results obtained with this test in May, 1930 were much lower for barren ewes than for ewes which had just lambed. In July they were very much alike. Both classes fell from July to October, but, while the figures for the ewes continued to fall from October to January, those for the hoggs and barren ewes rose considerably. The barren ewes fell slightly in March, but the hoggs and ewes gave the same average reading as in January. The ewes showed an in-:crease in titre in May, while hoggs remained steady. The gimmers, on the other hand, fell from October to January, rose in April and fell again in June.

Summary. The extent of individual variations noted among all animals sampled on one day ranged from 0.5 to 3.2 M.H.D.s per c.c. For sheep in the same heft sampled on one day these limits were 0.1 and 3.2 M.H.D.s per c.c. Individual animals differed in their response to this test at different times.

Among the ewes heft I tended to give the highest readings in this reaction during the October samplings, and in January there were no noteworthy differences In March heft III was highest and heft IV lowest on all days, but by May heft III had dropped to the lowest position, with heft I highest. In November heft II appeared to give the best response to the test, when all days were considered, while heft IV was lowest.

The hoggs showed no heft differences in October, but in February there

were/

were indications that heft I was highest. No further differences appeared among these animals.

Heft II was highest for gimmers and heft I for barren ewes in February, while in March the highest figures for these classes of animals were given by hefts III and I respectively.

Examination of the mean response given by the different hefts at the various periods of sampling elicits the fact that the fall in haemolysin content of the sera of ewes which occurs throughout the winter appears to have been checked earlier in hefts II and III in 1931, and prevented altogether in 1932. This fall did not appear in hoggs' sera until January, and was re-:tarded in Hefts II and III till March. It occurred in all hefts in May.

Titration of Complement.

As this titration was first included in the reactions to be tested in July, 1931, ewes are the only type of animal to which it has been applied.

Individual Variation. The variation in this test is considerably greater than in the haemolytic reaction with rabbit erythrocytes. Because of the daily variation a large range of M.H.D.s per c.c. had to be covered in this test, and for reasons of economy of time and serum the quantities were fairly widely spaced. Those quantities usually tested against 0.25 c.c. of sensitized corpuscles/

TABLE

HAEMOLYTIC REACTION

DAILY

Ewes.

	1930. May. 27.	Jly. 15.	Oct. 13.	15.	22.	1931. Jan. 19.	21.	26.
Heft I.	1.1	2.8	2.7	2.5	1.8	1.5	2.1	2.3
II.	1.3	3.1	2.5(4)	2.3	2.1	1.3	1.9	2.1
III.	1.4	2.8	2.5	2.3	2.0	1.2	2.1	2.1
IV.	1.3	2.9	2.2	2.2	2.1	1.3	1.9	1.8
Overall	1.3	2.9	2.5	2.3	2.0	1.3	2.0	2.0

Gimmers.

	1930. Oct. 27.	1931. Feb. 2.	Apl. 6.	8.	June. 2.
Heft I.	1.7(4)	1.4(4)	-	2.2(4)	2.2
II.	1.9	2.0	2.2	-	1.7
III.	2.1	1.5	3.0	-	1.6
IV.	1.8	1.4	-	2.0(6)	1.7
Overall	1.9	1.6	2.6	2.1	1.8

Month Avge 2.3

PERIOD AVERAGES

Ewes.

	1930. Oct.	1931. Jan.	Mar.	May.	Jly.	Nov.	1932. Mar.
Heft I.	2.3	1.9	1.8	3.1	3.2	2.4	2.9
II.	2.4(14)	1.8	1.8	2.8	2.5	2.5	2.7
III.	2.2	1.8	2.1	2.6	2.5	2.4	2.7
IV.	2.1	1.6	1.4	2.9	2.7	2.2	2.8
Overall.	2.2	1.8	1.8	2.9	2.7	2.4	2.8
	(15)	(20)	(20)	(20)	(10)	(35)	(30)

(The figures in brackets below the period averages indicate the number of results from which those averages are calculated).

XXIII.

WITH RABBIT ERYTHROCYTES.

AVERAGES. (M.H.D.s per c.c.)

	Mar. 28.	23.	25.	30.	Apl. 1.	May. 21.	23.	27.	29.	Jly. 20.
	1.7	1.8	2.1	-	1.7(10)	3.4	3.6	2.4	3.2	2.4
	1.9	1.7	2.0	1.7(10)	-	3.0	3.2	2.3	2.7	2.3
	1.7	2.1	2.1	2.0(10)	-	2.4	2.9	2.1	3.0	2.3
	1.4	1.6	1.5	-	1.3(10)	2.7	3.0	3.1	2.8	2.9
	1.7	1.8	2.0	1.8	1.5	2.9	3.2	2.5	2.9	2.5

Hoggs.

	1930. Oct. 27.	Nov. 6.	1931. Feb. 4.	11.	Apl. 6.	8.	13.	15.	June. 4.	8.
	1.8	1.6	2.5	3.2	-	2.4	-	2.7	1.8	2.2
	1.7	1.7	2.3	2.2	1.9	-	3.1	-	1.8	2.6
	1.8	1.7	2.4	2.5	2.5	-	-	2.7	2.0	2.0
	1.7	1.6	2.3(4)	2.6	-	2.8(4)	2.4	-	2.0	2.0(4)
	1.7	1.6	2.4	2.6	2.2	2.3	2.7	2.7	1.9	2.2

Hoggs.

	1930. Oct.	1931. Feb.	Apl.	June.
	1.7	2.9	2.5	2.0
	1.7	2.2	2.5	2.2
	1.7	2.4	2.6	2.0
	1.6	2.5(9)	2.5(9)	2.0(9)
	1.7	2.6	2.6	2.2
	(10)	(10)	(10)	(10)

22.	Nov. 2.	5.	9.	11.	17.	18.	23.	25.	1932. Mar. 21.	23.	28.	30.	Apl. 11.	13.
3.9	2.6	2.5	1.7	-	2.5	2.0	2.0(4)	3.0(6)	3.6	2.6	2.4	3.1	2.9	2.7
2.7	2.2	2.6	1.9	-	2.6	2.5	2.0	3.6	2.5	2.0	2.3	3.1	3.3	3.1
2.6	2.4	2.4	2.0	-	2.1	2.3	2.2	3.5	2.8	2.0	2.7	3.0	3.0	2.8
2.5	1.9	2.0	1.8	-	1.9	2.3	2.4	3.2	3.0	2.2	2.6	2.9	3.3	2.7
3.0	2.3	2.4	1.9	-	2.3	2.3	2.2	3.3	3.0	2.2	2.5	3.0	3.1	2.8

Barren Ewes.

1930. May 7.	Jly. 7.	Nov. 3.	1931. Feb. 9.	Apl. 13.	15.
2.3	2.6	1.5	3.8	-	3.1
2.3	2.3	2.0	2.9	2.7	-
2.3	3.2	2.0	3.6	-	2.7
2.9	2.3	2.1	3.4	2.2	-
2.4	2.6	1.9	3.4	2.4	2.9

Month Avge.
2.7

corpuscles were 0.025, 0.05, 0.1, and 0.15 c.c., which would translate into 20, 10, 5, and 3.3 M.H.D.s per c.c. for 0.5 c.c. of sensitized corpuscles. Intermediate readings of 13.3, 6.6, 4.0 and 2.8 M.H.D.s per c.c. were also used as explained on page 15 . During the first period that this test was in use still greater numbers of M.H.D.s per c.c. were observed, necessitating the use of smaller quantities of serum in the titration. The higher readings which could be made then were 40.0 and 25.0 M.H.D.s per c.c.

In July the individual variations were from 40.0 to 16.6 M.H.D.s per c.c. In July as the number of M.H.D.s per c.c. observed became less, the variations grew smaller, the greatest occurring on any one day being between 20.0 and 5.0 M.H.D.s per c.c. Variation of this extent was also observed in one heft on that day. These were also the limits of the greatest variation on one day observed during the March, 1932 samplings, while 20.0 and 0.6 were the limits within one heft.

Since only two animals in each heft have been bled at all three periods of sampling it is impossible to estimate any difference in periodic response of individuals to this reaction.

Heft Differences. On the two days on which this reaction was tested in July, 1931, the order of the hefts was entirely different. Though the same order was maintained on the first two days of the November sampling, during the rest of that period and in the following March no consistency in behaviour was observed.

Table XXIV.

The period averages for the different hefts show the highest complement

titre/

titre in heft III at all periods. All hefts behave similarly from period to period.

Summary. Though the daily averages show no consistency in the relative positions maintained by the hefts during any period, yet the mean for each complete period is highest in heft III, and in November and March lowest in heft II. No difference in the behaviour of the hefts from period to period appears.

Agglutination Reaction with B. paratyphosus B.

Individual Variation. The highest and lowest results recorded in one day's sampling usually differed from each other by three or four agglutination units. Occasionally the difference was only one or two units, and twice it was five units (once among the ewes in March, and once among the gimmers in the same period of sampling). Among the ewes the most frequent variation within one heft was two agglutination units, but one and three units were quite frequently observed, and on one occasion each, four and five units of difference between the highest and lowest results in a heft were noted. Among the hoggs one unit only of difference was observed almost as frequently as two units, and four units was the greatest difference noted.

Differences between Hefts and between Types of Animals - Ewes. (Table XXV). During the preliminary tests heft III gave the highest results in May, and heft II in July. In the following October the order of the hefts on the various days of sampling was:-

TABLE XXIV.

TITRATION OF COMPLEMENT.

DAILY AVERAGES.

(M.H.D.s per c.c.)

Ewes.

	1931. Jly. 20.	22.	Nov. 2.	5.	9.	17.	18.	22.
Heft I.	-	19.0	6.6	6.3	4.4	6.0	6.6	18.3(4)
II.	17.5	23.6	8.3	8.6	4.3	4.2	6.6	9.3
III.	20.9	23.6	7.6	7.0	4.8	6.4	8.3	13.3
IV.	21.0	18.7	5.3	5.8	3.8	7.3	8.6	10.6
Overall	19.8	21.2	7.0	6.9	4.3	6.0	7.6	12.6

Ewes (Ctd.)

	1931. Nov. 25.	1932. Mar. 21.	23.	28.	30.	Apl. 11.	13.
Heft I.	5.3(6)	7.5	4.8	10.7	10.6	7.3	4.3
II.	7.6	10.0	4.8	9.3	6.6	7.0	4.2
III.	8.6	17.3	4.6	10.7	7.3	6.3	4.3
IV.	12.0	12.6	4.4	9.3	9.0	6.3	4.9
Overall.	8.2	11.8	4.6	10.0	8.4	6.7	4.4

PERIOD AVERAGES.

Ewes.

	1931. Jly.	Nov.	1932. Mar.
Heft I.	19.0(5)	7.3	7.5
II.	20.6	7.0	6.9
III.	22.2	8.0	8.4
IV.	19.8	7.6	7.8
Overall.	20.6	7.5	7.7
	(10)	(35)	(30)

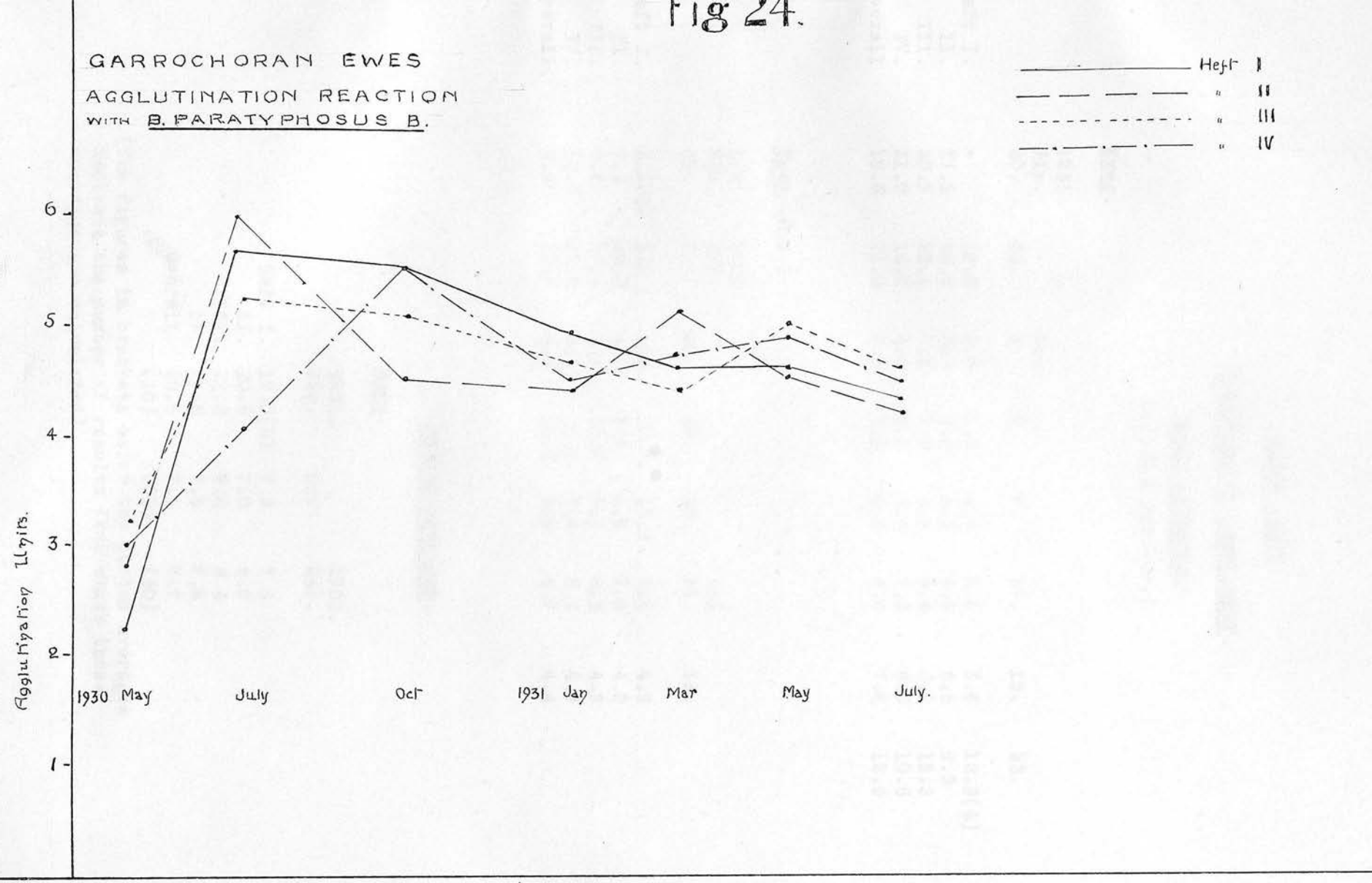
(The figures in brackets below the period averages indicate the number of results from which those averages are calculated).

Fig 24.

GARROCHORAN EWES

AGGLUTINATION REACTION

WITH B. PARATYPHOSUS B.



October 13th	I > IV > III > II.
" 15th	I > II > III = IV.
" 22nd	IV > I > III > II.
Whole period	I = IV > III > II.

Heft I occupied either first or second position on all days while heft IV also was high on two days. Hefts II and III were definitely lower than the other hefts on the first and last days. Again in January on three out of the four days heft I was highest but on the last day in this period it was lower than the other three hefts. Heft III also tended to keep at the higher end of the scale. The order of the hefts as determined by the average figure over the complete period was I > III > IV > II. In March, May and July the hefts which were highest one day would be low the next and vice versa, though in March heft II maintained a fairly high position on all days, as did heft III on three days in July. This test was discarded after July, 1931.

Hoggs. In October heft IV tended to be highest (i.e. it was first along with heft II one day and second the next day). No differences can be noted at the samplings in February and April, but in June heft II gave indications of being higher than the others.

Gimmers. In February heft I was somewhat higher than the other hefts but no further differences appeared to be present among these figures.

Barren Ewes. Heft I gave the highest results in the preliminary test in July, 1930 and again the following February. Otherwise nothing even of apparent significance can be seen.

The curves of the means of each period for each heft are shown in Figure 24. Heft I rose from May to July, 1930 then fell gradually until the last period of sampling in the following July. Heft II also rose from May to July, fell/

fell sharply to October, and more slowly to January, rose somewhat in March, then fell through May to July, 1931. Heft III behaved in a different manner, rising from May to July, 1930, then falling until March, 1931. A slight increase in May was followed by a final drop in July. Heft IV showed yet another type of behaviour, rising until October then falling in January, i.e. it showed a further rise from July to October when in all other hefts a fall in reaction was recorded. From January this heft rose again till May, then fell in July. Hefts I and III ran parallel except between March and May, 1931, but hefts II and IV showed no similarity either to each other or to hefts I and III.

Among the hoggs all hefts decreased in activity from October to February. Heft I increased again in April and finally fell in June. Hefts II and III decreased further in April, and increased slightly in June, while heft IV remained steady in April, then fell in June.

No differences appear in the behaviour of the hefts among the gimmers. In the preliminary tests in July, 1930 heft I of the barren ewes was much higher than the other hefts. It then fell to the level of the other hefts in October; all hefts showed a decrease in activity at this time. In February hefts III and IV showed a further slight decrease, heft II remained steady and heft I increased.

The activity of the ewes' sera in this reaction was considerably higher throughout than that of the hoggs and the barren ewes, but similar to that of the gimmers.

Summary./

Summary. The limits of variation among individuals sampled on one day was from two to five agglutination units and among individuals within one heft sampled on one day from nil to five units.

During no period of sampling did any heft maintain the same position relative to the other hefts on all days. In the various months the follow-:ing hefts occupied a higher position than the others on a majority of the sampling days:-

Ewes.	October, I and IV;	January, I and III;
	March, II;	July, III.
Hoggs.	October, IV;	June, II.
Gimmers.	February, I.	
Barren Ewes.	July, 1930. I;	February, I.

Comparing the behaviour of the different hefts throughout the fourteen months May, 1930 to July, 1931, it is seen that hefts I and III run parallel except between March and May, 1931. Hefts II and IV behave entirely independently. The various hefts of hoggs showed different types of behaviour in April; heft I rose from the level of its activity at the preceding sampling, heft IV remained steady, and hefts II and III fell. From April to June hefts I and IV fell and hefts II and III rose slightly.

The activity of the ewes and gimmers' sera were similar at all times and considerably greater than that of the hoggs and barren ewes.

Agglutination Reaction with B. abortus (Hog).

Individual Variation. The variation among ewes for day to day samplings, irrespective of heft, ranged from one to six units, the most frequent being three or four units. Among those in the same heft, the range for different days was from nil to six units, and the variation appearing most frequently

was/

TABLE

AGGLUTINATION REACTION

DAILY

Ewes.

	1930. May 27.	Jly. 15.	Oct. 13.	15.	22.	1931. Jan. 19.	21.	26.
Heft I.	2.2	5.6	5.2	6.0	6.2	6.2	4.4	4.4
II.	2.8	6.0	3.4	5.4	4.8	5.0	4.0	3.8
III.	3.2	5.2	4.6	5.0	5.6	6.0	3.8	4.0
IV.	3.0	4.0	4.8	5.0	6.6	6.0	3.4	3.8
Overall.	2.9	5.2	4.5	5.3	5.8	5.8	3.9	4.0

Gimmers.

	1930. Oct. 27.	1931. Feb. 2.	Apl. 6.	8.	June. 2.
Heft I.	5.7(4)	5.0(4)	-	5.0(4)	4.8
II.	5.6	4.4	4.4	-	4.0
III.	5.8	4.4	3.6	-	4.6
IV.	5.6	4.6	-	4.7(6)	5.0(1)
Overall	5.7	4.6	4.0	4.8	4.5

Month Avge 4.4

PERIOD AVERAGES

Ewes.

	1930. Oct.	1931. Jan.	Mar.	May.	July.
Heft I.	5.5	4.9	4.6	4.6	4.3
II.	4.5	4.4	5.1	4.5	4.2
III.	5.1	4.7(19)	4.4(19)	5.0	4.5
IV.	5.5	4.5	4.7	4.9	4.4
Overall	5.2	4.6	4.7	4.8	4.3
	(15)	(20)	(20)	(20)	(10)

(The figures in brackets below the period averages indicate the number of results from which those averages are calculated).

XXV.

WITH B. PARATYPHOSUS B.

AVERAGES. (in agglutination units)

28.	Mar. 23.	25.	30.	Apl. 1.	May. 21.	23.	27.	29.
4.6	4.6	5.4	-	4.3(10)	4.2	5.0	4.2	5.2
4.8	5.8	5.2	4.8(10)	-	4.8	4.8	3.6	5.0
5.2(4)	5.2	4.4	3.9(9)	-	4.8	5.4	4.8	5.0
5.0	5.2	4.6	-	4.5(10)	4.6	5.6	4.6	4.8
4.9	5.2	4.9	4.4	4.4	4.6	5.2	4.3	5.0

Hoggs.

	1930. Oct. 29.	Nov. 6.	1931. Feb. 4.	11.	Apl. 6.	8.	13.	15.	June. 4.	8.
	5.4	2.8	3.8	3.4	-	4.6	-	3.6	3.4	3.2
	5.6	2.8	3.4	4.6	3.4	-	3.4	-	3.6	3.6
	4.8	3.6	3.2	4.8	3.2	-	-	3.2	2.2	3.4
	5.6	3.4	3.2(4)	4.6	-	3.7(4)	4.2	-	3.2	3.7(4)
	5.3	3.1	3.4	4.3	3.3	4.2	3.8	3.4	3.1	3.5

Hoggs.

1930. Oct.	1931. Feb.	Apl.	June.
4.1	3.6	4.1	3.3
4.2	4.0	3.4	3.6
4.2	4.0	3.2	2.8
4.5	4.0(9)	4.0(9)	3.4(9)
4.2	3.9	3.6	3.3
(10)	(10)	(10)	(10)

Jly. 20.	22.
5.0	3.6
4.2	4.2
4.2	4.8
4.2	4.6
4.4	4.3

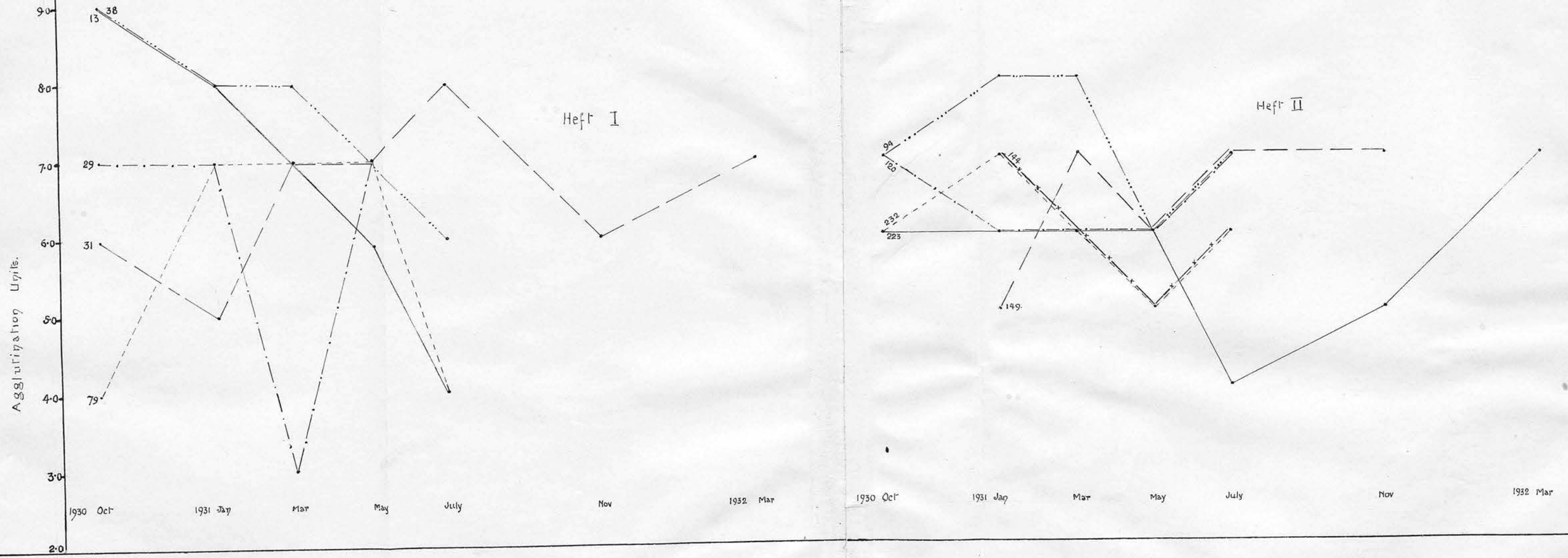
Barren Ewes.

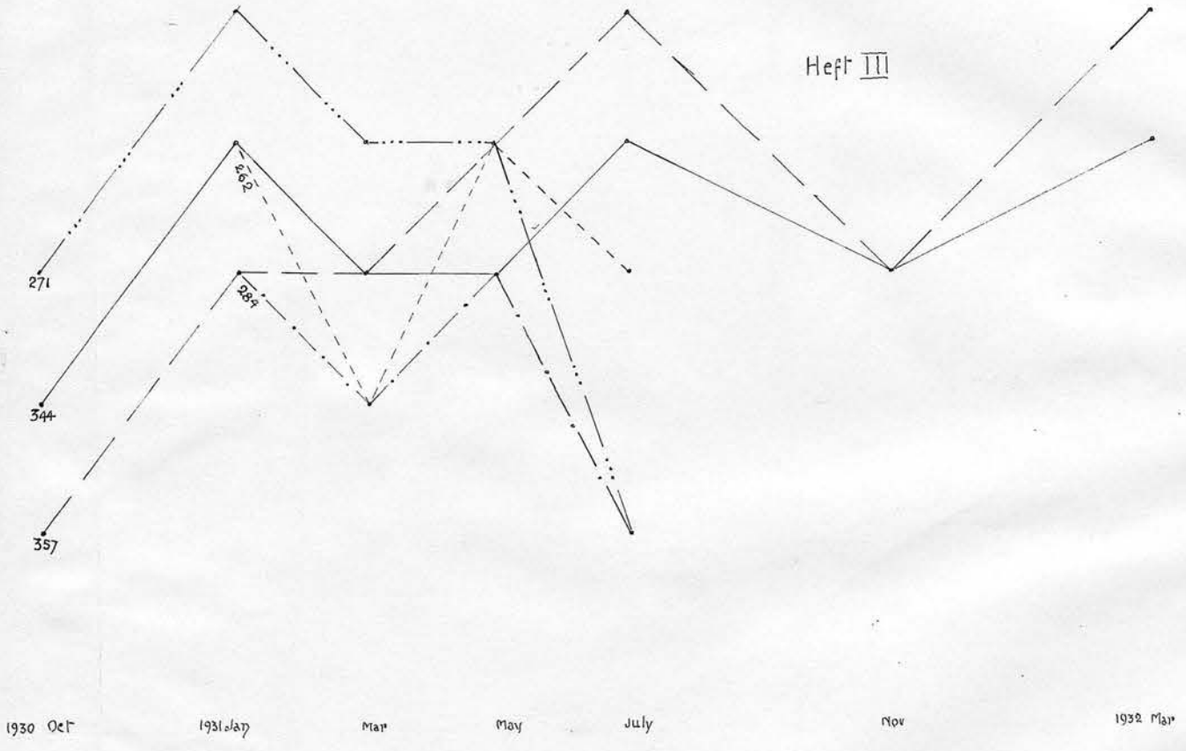
1930. May. 7.	Jly. 7.	Nov. 3.	1931. Feb. 9.	Apl. 13.	15.
3.0	6.4	3.8	4.8	-	4.0
3.3	4.5	3.4	3.6	4.0	-
3.0	5.3	4.2	3.6	-	3.8
3.0	5.0	4.4	3.8	4.0	-
3.1	5.4	3.9	3.9	4.0	3.9

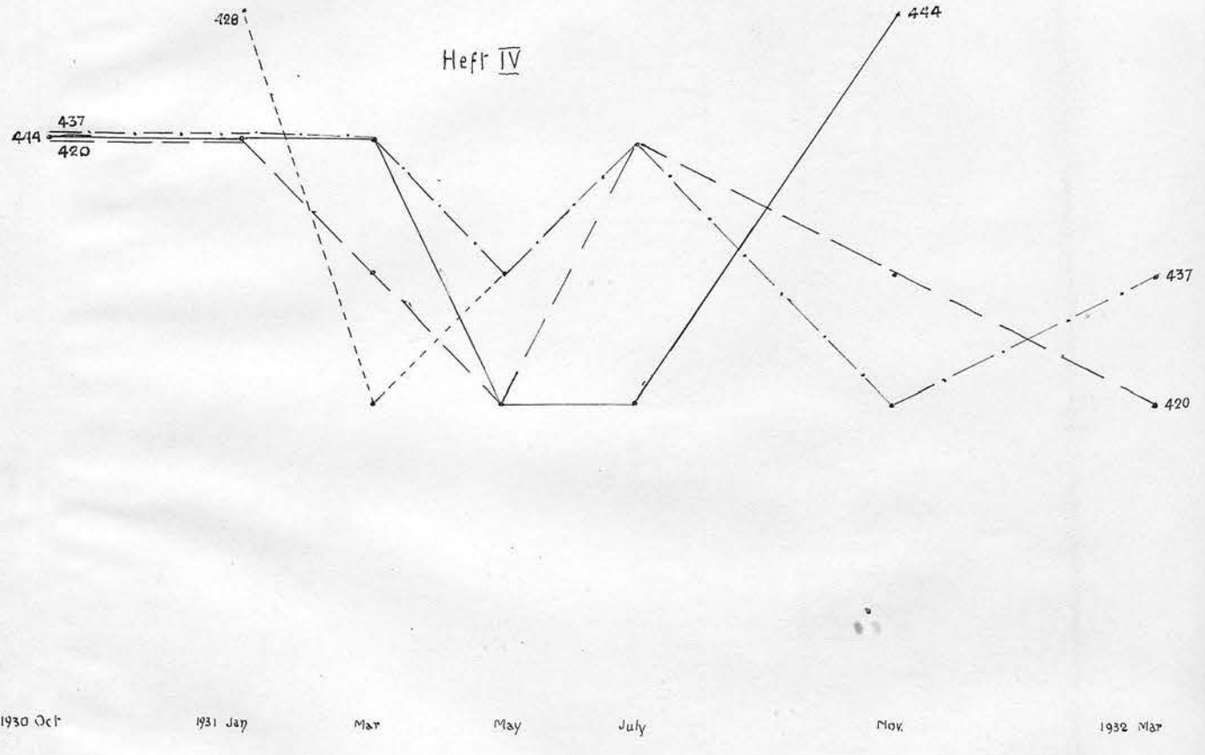
Month Avge
3.9

Fig 25.

AGGLUTINATION REACTION
WITH B. ABORTUS (HOG).




Heft III
271
462
344
284
357
1930 Oct
1931 Jan
Mar
May
July
Nov
1932 Mar


Heft IV
428
444
437
414
420
437
420
1930 Oct
1931 Jan
Mar
May
July
Nov.
1932 Mar

one or two, then three units. Equally large variations occurred at all periods of sampling, and also among the other types of animals.

Periodic Response of Individuals. (Fig.25). As with the reactions previously discussed, individuals vary exceedingly in their response to this test, and it is almost impossible to find two animals which will give parallel curves when the mean reactions for each period are plotted.

Differences between Hefts and between different Types of Animals.(Table XXVI). The differences between the hefts during the preliminary tests were very small. In October the mean readings for hefts I, II and IV were the same on the first and second days. The reading for heft III was much lower on the first day than on the second. In January the order of the hefts on different days varied, but heft I showed the highest agglutination titre on three days, while heft IV was highest on the fourth day and second to I on two others. Heft III was lowest each day. In March heft I was highest on the two days on which all hefts were sampled, and heft III was second.

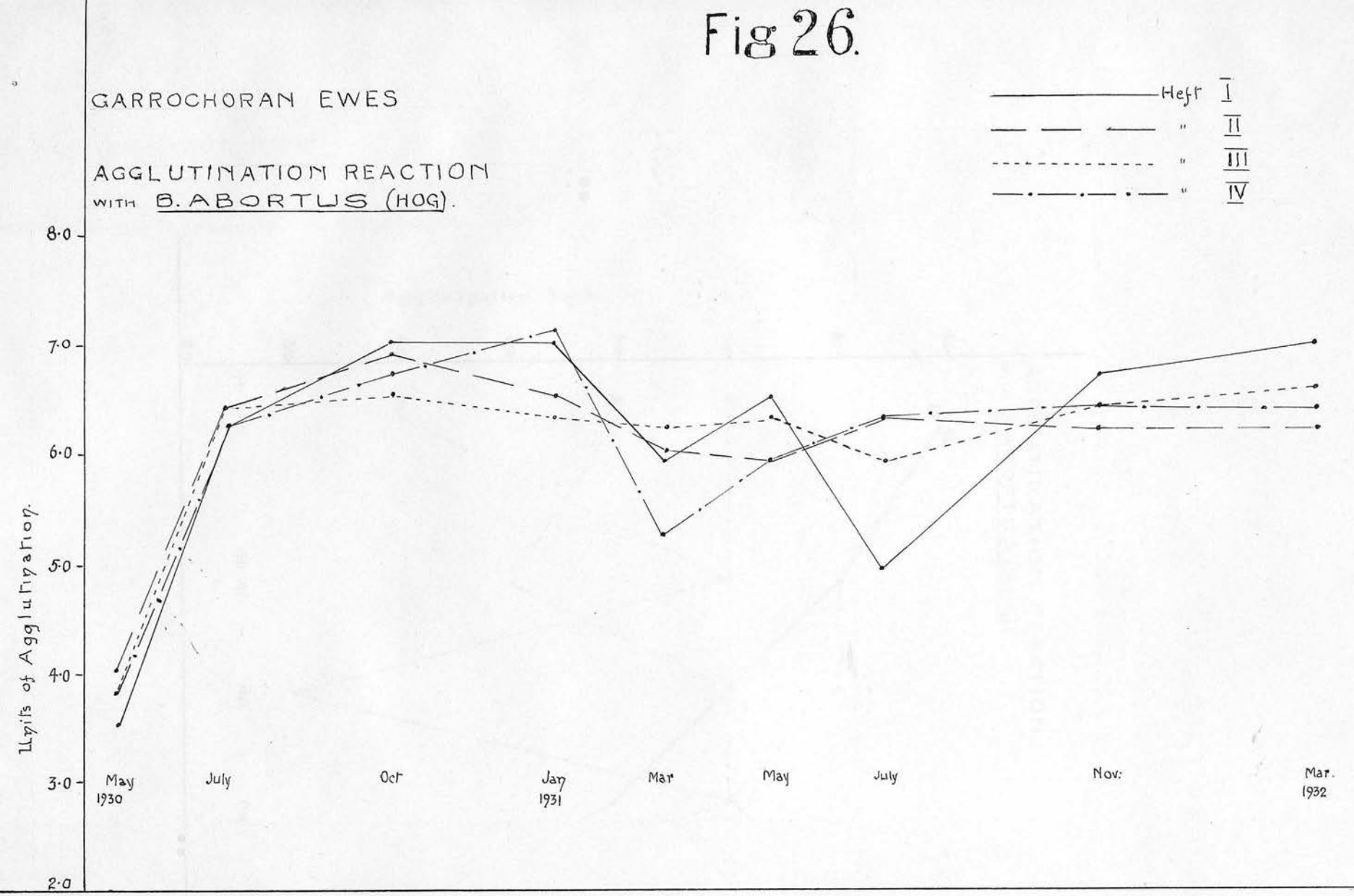
In May a different heft was highest each day, and in July hefts II and IV were highest and heft I lowest. By November heft I had returned to the higher end of the scale, and heft II to the lower end; and in March, 1932 heft I was highest on all days while heft IV was equal to it on two days and heft III on one. Heft II remained low throughout this period of sampling.

Hoggs. This test was only carried out on one day during the October-November and January-February samplings. In October the order was II, > I, >IV, >III, and in February, I, >IV, >II, >III. In June heft I was higher than the other/

Fig 26.

GARROCHORAN EWES

AGGLUTINATION REACTION
WITH B. ABORTUS (HOG).



other hefts, which were much alike.

Barren Ewes. Heft II was highest during the preliminary tests in May, 1930, and in July hefts I and II were considerably higher than hefts III and IV. In November the order of the hefts was I,> IV,>II,>III, and in February, III, >(I and IV),>II.

Gimmers. In June the figure for heft IV was lower than the figures for the other hefts. This was also the case in April but the sampling of the hefts on different days make it difficult to assess this difference.

If the daily averages for the separate hefts are examined, it will be seen that a greater variation occurs in hefts I and IV than in hefts II and III. Of a total of thirty-one daily observations, five and three, in hefts II and III respectively, fall outside the limits of 7.0 and 5.5 units, while the corresponding numbers in hefts I and IV are twelve and nine. From July,1930 to March, 1931, the periodic means for hefts II fell within the limits 5.9 and 6.9 units and for heft III, within the limits 6.5 and 5.9 units. The correspond-:ing limits for hefts I and IV were 7.0 and 4.9, and 7.1 and 5.2 units respect-:ively. This is the most outstanding point to be noted in figure 26 where the period averages for the different hefts are charted. The figures in May,1930 are much lower than those observed at any subsequent time.

Hefts I and IV of the hoggs show a drop in mean agglutination titre from February to April, followed by a rise in June. Heft III behaved in exactly the opposite manner. Heft II fell from October to February, rose again to its October level in April, and fell again in June. Among the barren ewes and gimmers/

gimmers no discrepancies appeared in the behaviour of the hefts, each rising and falling at the same time as the others.

In October and January the period averages for the ewes were higher than the corresponding figures for the hoggs, but in the March-April period of sampling, and again in May and June, this difference had disappeared. At all periods of sampling except October, the mean agglutination titre for the barren ewes was considerably higher than for the breeding ewes. In February and April the figure for gimmers was much lower than for any other type of animal, but in June it was comparable to those for ewes and hoggs.

Summary. A considerable range of variation between individuals has been observed, extending from one to six units among animals of all hefts sampled on one day, and from nil to six units among animals of one heft sampled on one day. The variations noted most frequently were three or four units in the former case, and one or two units in the latter. There is no parallelism between the curves obtained from different individuals when their titres at different samplings are plotted out.

While the order of the hefts of ewes in January, according to the average titre over that period, was (I and IV)>(II and III), that of the hefts of hoggs was I, IV, II, III. The order for the ewes in March, taking into account only the two days on which all hefts were sampled, was I,>III,>IV,>II. The order of the hefts of barren ewes at this time was III,>IV,>I,>II. It is not possible to assess the order for the hoggs at this time, but at the May-June sampling it was I, >(II, III, and IV). The hefts of ewes varied in their relative positions from day to day at this sampling, but by July they were in the order (II and IV) >III,>I. By November this was almost completely reversed/

reversed again, being I,> (III and IV),> II, which changed to I,>III,>IV,>II, in the following March.

Hefts II and III of the ewes vary less from period to period than do hefts I and IV. While the agglutination titre of hefts I and IV of the hoggs fell from February to April and rose again in June, that of heft III behaved in exactly the contrary manner.

The mean agglutination titre for the barren ewes was higher than that for any other type of animal at all samplings except October when it was lowest. In October and January, figures for breeding ewes were higher than for hoggs, but in the two subsequent samplings this difference had disappeared. The figures for gimmers were lower than those for other types in February and April, but comparable with those for ewes and hoggs in June.

Garrochoran Sheep Wintered at Aberdeen.

During the winter 1930-31 hoggs and ewes were sent from Garrochoran to Aberdeen. The blood of these sheep was sampled immediately after the Garrochoran samplings in October, 1930, January and March, 1931. They were returned to Garrochoran in April and sampled there in May. The results obtained from them are given in table XXVII.

Garrochoran Hoggs and Ewes at Aberdeen.

		Agglutination Reaction with			Bactericidal Reaction with		
	Haemolytic Reaction.	B.paratyphosus B.	B.abortus	B.suipestifer	B.coli"X"	Str.*
Hoggs. 18/11/30.	2.2	4.7	6.4	4.3	0	-
Ewes. 13/11/30.	2.8	2.7	4.4	4.8	0	1.8
Hoggs. 16/2/31.	2.6	2.9	4.1	4.1	0	0.3
Ewes. 18/2/31.	3.2	4.5	6.2	2.0	0	0.2
Hoggs. 20/4/31.	2.4	3.0	5.6	4.0	0.6	-
Ewes. 22/4/31.	3.2	4.4	5.6	4.2	0.3	0.3

These/

* Str. = Streptococcus haemolyticus

TABLE

AGGLUTINATION REACTION

DAILY.

XXVI.

WITH B. ABORTUS (HOG)

AVERAGES. (in agglutination units)

Ewes.

	1930 May 27.	Jly. 15.	Oct. 13.	15.	1931. Jan. 19.	21.	26.	28.
Heft I.	3.5	6.2	7.0	7.0	7.0	7.0	6.0	8.0
II.	4.0	6.4	7.0	7.0	6.6	6.4	6.2	6.8
III.	3.8	6.4	6.0	7.0	6.6	6.4	6.0	6.5(4)
IV.	4.0	6.2	6.6	6.6	6.8	6.4	7.0	7.8
Overall	3.6	6.2	6.6	6.9	6.7	6.5	6.3	7.3

Gimmers.

	1930. Oct. 27.	1931. Feb. 2.	Apl. 6.	8.	June. 2.
Heft I.	-	3.0(4)	-	5.7(4)	6.2
II.	-	3.6	5.2	-	6.4
III.	-	3.6	5.2	-	6.4
IV.	-	3.4	-	4.1(6)	5.8
Overall	-	3.5	5.2	4.8	6.2

Month Avge 5.0

Hoggs.

	1930. Oct. 29.	Nov. 6.	1931. Feb. 4.	11.	Apl. 6.	8.	13.	15.	June. 4.	8.
	-	6.4	6.6	-	-	5.6	-	6.0	6.4	6.2
	-	6.6	5.8	-	5.6	-	7.6	-	5.6	5.8
	-	5.6	5.6	-	6.0	-	-	6.0	5.6	5.8
	-	6.2	6.0(4)	-	-	4.0(4)	6.6	-	5.8	5.7(4)
	-	6.2	6.0	-	5.8	4.9	7.1	6.0	5.8	5.9

PERIOD AVERAGES.

Ewes.

	1930. Oct.	1931. Jan.	Mar.	May.	July.	Nov.	1932. Mar.
Heft I.	7.0	7.0	5.9(19)	6.5	4.9	6.7	7.0
II.	6.9	6.5	6.0(19)	5.9	6.3	6.2	6.2
III.	6.5	6.3(19)	6.2	6.3(17)	5.9	6.4	6.6
IV.	6.7	7.1	5.2	5.9(19)	6.3(9)	6.4	6.4
Overall	6.7	6.7	5.8	6.1	5.8	6.5	6.5
	(10)	(20)	(20)	(20)	(10)	(40)	(30)

(The figures in brackets below the period averages indicate the number of results from which those averages are calculated).

Hoggs.

	1930. Oct.	1931. Feb.	Apl.	June.
	6.4	6.6	5.8	6.3
	6.6	5.8	6.6	5.7
	5.6	5.6	6.0	5.7
	6.2	6.0(4)	5.4(9)	5.8(9)
	6.2	6.0	6.0	5.9
	(5)	(5)	(10)	(10)

Nov.								1932. Mar.				Apl.	
2.	5.	9.	11.	17.	18.	23.	25.	21.	23.	28.	30.	11.	13.
6.8	6.8	6.2	7.6	5.8	7.2	7.2(4)	6.3(6)	7.6	6.0	7.2	7.4	6.6	7.2
6.0	5.2	6.0	7.4	6.4	6.8	6.4	6.0	6.2	4.6	7.0	7.2	5.6	6.4
5.8	5.8	7.0	7.4	6.2	6.2	6.6	6.4	7.2	6.0	6.8	6.2	6.4	7.0
6.0	5.4	6.4	7.8	5.2	6.8	7.0	6.6	6.6	5.6	7.2	7.0	6.6	5.2
6.1	5.8	6.4	7.5	5.9	6.7	6.8	6.3	6.9	5.5	7.0	6.9	6.3	6.4

Barren Ewes.

1930.			1931.		
May	Jly.	Nov.	Feb.	Apl.	
7.	7.	3.	9.	13.	15.
4.2	7.4	6.6	7.8	-	6.4
6.2	7.2	4.8	7.0	7.6	-
4.3	6.6	4.4	8.0	-	6.0
5.3	6.3	5.8	7.8	7.4	-
5.0	7.0	5.4	7.6	7.5	6.8

Month Avge
6.8

These figures may be compared with corresponding mean values from sheep at Garrochoran (table XIX). The serum of the ewes at Aberdeen showed the higher haemolytic activity at all periods of sampling. The reactions with B.suipestifer and B. coli "X"were stronger in the same sheep in November and April but the positions were reversed in January-February. The Garrochoran sheep showed the higher agglutination titres to B. paratyphosus B.and B. abortus in November but the results from both lots were approximately equal in February and April.

In the case of the hoggs, the sheep at Aberdeen again showed the higher haemolytic activity in November, but in February and April the animals at both places were almost equally active. The reaction with B.suipestifer was stronger with the sera from the animals at Aberdeen at all times. In direct opposition to the results obtained from the ewes, the reaction with B. coli "X" was stronger from the Garrochoran animals in November and February, but from the animals at Aberdeen in April. The agglutination titres to B.paratyphosus B. were similar in both sets of animals at each period of sampling, as were those to B. abortus in November and in April. In the latter case they became considerably higher in the Aberdeen hoggs in February. Unfortunately, these results, so far, have not been analysed statistically.

Summary of Garrochoran Experiment:

Here, as in the case of the Ashtown experiment, it is difficult to interpret the results obtained. More complicating factors arise than in the previous experiment, e.g. meteorological conditions, pregnancy and lactation, in addition to the nutritional problem under investigation, while variations in the level of the reactions are to be seen from period to period of sampling and even from day to day within each period.

The/

The first point of interest which appeared on the commencement of this experiment was the markedly lower level of the results as compared with those obtained from the Ashtown animals. Comparison of black-faced ewes at Garrochoran with others of the same breed and at the same stage of lactation, but in better condition, at Aberdeen showed that the difference was not due to breed alone. Border-Leicester ewes at Aberdeen showed somewhat higher results in the haemolytic reaction than did the black-faced ewes at the same place, indicating that difference of breed may have some effect on the antibody content of the serum.

A difficulty was encountered when it became apparent that considerable variations were occurring in all reactions from day to day. These variations were common to all animals tested on one day and therefore appear to be due to some external factor. The technique has been kept as strictly uniform as possible and it was thought that these differences might be due to meteorological changes or the conditions imposed upon the animals previous to drawing the blood sample, or to conditions of transport of the sample after withdrawal from the animal body. Experiments carried out with a view to determining whether these factors might cause any change in the antibody content of serum have been unsuccessful so far in eliciting any reason for the daily variations. The different reactions varied independently of each other for the most part, though latterly it was found that a certain parallelism occurred between the curves when the mean daily results were plotted for the titration of complement, the bactericidal reaction with Streptococcus haemolyticus and the agglutination reaction with B. abortus (Hog). A similar parallelism existed in the case of the bactericidal reaction with B. coli "X" and the haemolytic reaction with

rabbit/

rabbit erythrocytes, but they were entirely unlike the three already mentioned. There was one day on which very low figures were recorded in all reactions.

Individual variations appeared on all days on which reactions were tested, but were more marked on some days than on others. The bactericidal reaction with B. suipestifer shows this variation to only a slight extent, but it is more definite in the haemolytic and agglutination reactions.

Few heft differences appeared throughout this experiment. It was very seldom that the hefts maintained any consistent positions relative to one another on all days in one period. More frequently one heft might be highest or lowest of the four on the majority of the days, while the other hefts changed their positions on the different days.

Among the ewes in March, 1931 heft III was highest in its response to the haemolytic reaction and the bactericidal reactions with B. suipestifer and Streptococcus haemolyticus. In the following May this heft had become lowest in the test with Str. haemolyticus but was highest in the bactericidal reaction with B. coli "X". Heft I took the place of III in the Str. haemolyticus reaction. No differences appeared in July. By November heft III showed comparatively high results in the bactericidal reaction with Str.haemolyticus and the titration of complement. At this time heft II was the least active in these two tests and also in the agglutination reaction, but showed a high order of activity in the haemolytic reaction and the bactericidal reaction with B. coli "X". Heft I was low in the reaction with B. coli "X" and high in that with B. abortus, but otherwise it was intermediate between II and III. Heft IV was of the same order of activity as heft II in the reaction with B./

B. coli "X" but lowest in the haemolytic reaction, though here any differences were exceedingly small. The order of the hefts in the various reactions at this time was as follows:

Bactericidal Reaction with B. coli "X"	(IV > II) > (III > I)
" " " Str. haemolyticus	III > (I & IV) > II.
Haemolytic "	II > (I & III) > IV.
Titration of Complement.	III > IV > I > II.
Agglutination reaction with B. abortus	I > (III & IV) > II.

Here again may be seen the grouping of the reactions which was noted at this time in the daily variations.

In the following March heft II occupied a low position relative to the other hefts in the bactericidal reaction with B. coli "X", the titration of complement and the agglutination reaction with B. abortus. The differences in the reaction with Str. haemolyticus and the haemolytic reaction are negligible.

It is interesting to note that a fall in haemolysin content of the serum which occurred in all animals in the winter of 1930-31 was arrested sooner in hefts II and III than in hefts I and IV. In the latter hefts the recovery was not observed until the May samplings, but in the former ones it was apparent in March. During the summer the haemolysin content increased in heft I until it was higher than in any of the other hefts but with the coming of winter it fell again in this heft and in heft IV while hefts II and III remained steady. The recovery from the fall occurred earlier in the winter of 1931-32 than the previous one as it was seen in all hefts by March, but in II and III to only a very slight extent.

A/

A curious reversal of the order of the hefts occurred in the agglutination reaction with B. abortus in July. Throughout the year from March, 1931 to March, 1932 the order of the hefts as elicited by the mean reaction at the various samplings was the following:-

March, 1931	I > III > IV > II.
May "	I > III > (II & IV)
July "	(II & IV) > III > I.
November "	I > (III & IV) > II.
March, 1932.	I > III > IV > II.

Among the hoggs the only point which appeared to be of any significance was that heft I gave the highest results in the haemolytic reaction with B. coli "X" in February, 1931. At the same time the barren ewes in heft I were also the highest with regard to the haemolytic reaction, but it was heft II which occupied this place among the gimmers. In March heft I of the barren ewes and heft II of the gimmers were highest in this reaction, among their own type of animal.

Owing to the extent of the daily variations it is almost impossible to elicit information from the data regarding seasonal variation. Such information is really only to be obtained from heft IV as with the remainder of the animals any apparent seasonal difference might be the cumulative effect of the supplementary feeding - as appears in the haemolytic reaction. Only in this reaction and the titration of complement did seasonal variation of any significance appear to occur. In the latter case the results obtained in July, 1931 were very much higher than those obtained in the following November or March.

When the different types of animals are compared regarding the strength of reaction given by their sera, the results are as conflicting as in most

cases/

cases previously discussed. One or two points are quite definite, however. For example, in the agglutination tests with B. paratyphosus B.the figures for the ewes and gimmers were always higher than those for the barren ewes and hoggs. Again, in the agglutination test with B. abortus, the barren ewes at all samplings after October gave higher results than any other type of animal, and in January and March the gimmers gave the lowest figures. In October and January the results of this reaction were greater for the ewes than the hoggs while the reverse was noted in the bactericidal reaction with B. coli "X".

The ewes and hoggs showed entirely different behaviour in their response to the haemolytic reaction. The ewes as a whole exhibited a fall in the haemolysin content of their serum from October to January, from which recovery took place in March or May according to the hefts (the mean for all ewes does not show this recovery till May). In the case of the hoggs the haemolysin content was greater in January than in October, then became less in March or May in the same hefts in which the rise had occurred in the ewes (again the mean for all hoggs places the recovery in May).

Comparison of ewes and hoggs wintered at Aberdeen with others retained at Garrochoran showed little difference between them in most reactions. The ewes wintered at Aberdeen showed a higher haemolytic activity than those at Garrochoran in November, February and April. In the bactericidal reactions the Aberdeen sheep gave the higher results in November,and April, but the Garrochoran sheep gave the higher figures at the February sampling.

The hoggs at Aberdeen showed the higher bactericidal reaction with

B./

B. suipestifer at all periods of sampling, but after their return to Garrochoran this difference disappeared. No further evidence of difference in behaviour was noted.

Clinical observations were made throughout the experiment and the sheep were weighed regularly. During the winter of 1931 all sheep lost weight, those in heft III losing least, and those in heft IV most, while hefts I and II were intermediate. In summer all gained weight but the order of the hefts remained the same, i.e. III > (I & II) > IV. The less easily measured factors of condition, strength, activity and general health corresponded to the weight, as far as could be determined by experts. Table XXVIII (Mackie, Fraser, Finkelstein & Anderson, 1932), gives other observations made during 1931, which serve to throw light on the general state of health and productivity of the sheep.

Table XXVIII.

	Heft I.	Heft II.	Heft III.	Heft IV.
Number of ewes	89	156	138	73
Percentage mortality of ewes	11	11	11	24
" birth rate of lambs	83	79	82	67
" mortality of lambs	20	11	18	26
Average birth-weight of lambs in lbs.	6	6	7	6
" weight of lambs at weaning	50	49	52	48
" fleece-weight of ewes.	3.7	3.8	4.0	3.1

From this it is seen that heft III was superior in all respects to heft IV, while hefts I and II were intermediate. Further details regarding the economic aspect of the research have been published in a communication by Orr and Fraser. (1932.)

It was hoped at the outset of the experiment that some relationship might be found between the clinical findings and the results of the serological tests. These latter results have been so varied, however, that it is difficult, so far/

far, to correlate differences in the serum reactions with dietary factors. The results of the haemolytic reaction with ewes corresponds to some extent with the clinical observations, as do the results of the toxin tests, which are discussed later (p.101).

SECTION V.

STATISTICAL ANALYSIS OF RESULTS OF GARROCHORAN EXPERIMENT.

As previously explained the sheep dealt with in these experiments were divided into four groups, each being on a different diet (see p.39). In the case of any particular set of observations, 20 sheep, five for each ration, were examined on one day, so that when twenty sheep on each were sampled, the examinations extended over four separate days, spaced out over two weeks. Examination of the animals was made at intervals of roughly two months, i.e. in October, 1930, January, March, May, July, and November, 1931 and March, 1932.

It will thus be seen that at least four distinct factors may have influenced any particular observation, namely, the season of the year, the effect of diet, the peculiarities of the day (e.g. technique and uncontroll-:able influences) and finally, the specific peculiarities of individual animals. The last two factors are obviously incidental, whereas it is important to obtain as much information as possible about the first two. The problem is, therefore, by statistical analysis of the data to disentangle these four factors and, in particular, to ascertain what specific effects can be attributed to the first two factors.

Statistical Methods.

The most suitable and generally applicable method of approaching a problem of this nature, appears to be the method of analysis of variance elaborated by Fisher (1925).

It is unnecessary to explain in detail this method, but a brief account of the general principle may be given here. Two fundamental notions are involved/

involved-(1) variance and (2) degrees of freedom. Variance may be taken roughly as measuring the scatter of the observations about the mean. This scatter may be looked upon as being due to so many degrees of freedom or independent factors involved. In general the total number of degrees of freedom will be one less than the number of independent observations since the sum of the deviations about the mean is necessarily zero, so that the variat-:ions about the mean are not completely independent, but are connected by one relation. Now the total number of degrees of freedom can be divided up in experiments such as are dealt with here, so that we can say that so many are due to the day, so many to the heft and so many to the individual, etc. In the same way the variance also may be divided into corresponding parts, so that for each factor, e.g. hefts, we have so much variance with so many degrees of freedom. It then becomes a simple matter to find the average variance for each degree of freedom for all the factors taken together or for each factor separately, e.g. hefts or days. Now the method of variance analysis depends on a fundamental proposition which may be stated as follows:-
that if the population is really homogeneous (i.e. the factors under discuss-:ion do not really affect the results) the variance for each degree of freedom will be the same however it is calculated, or at least the values so obtained will not differ significantly from each other. In practise then one calculates the variance for one factor, e.g. from the heft variations, and also, say, from another factor or from all the factors put together. Then, by a simple method which involves the use of the 'z' figure, calculated by Fisher and found in his book, one tests whether these two values differ significantly from each other. If they do, one is justified in concluding that the differences apparently/

apparently produced by the factor under discussion are not simply the result of individual variations in the animals, but that the factor has really produced a significant difference.

As an example the system mentioned above may be considered in which there are five animals in each heft and four hefts in each day and four days of sampling in a period. There are then 80 observations in each period giving 79 degrees of freedom. There is 1 mean for each day, therefore the effect of the days gives 3 degrees of freedom and there are 76 degrees of freedom due to the individual variation factor within the days. Of these 19 come from each day. On each day there are 4 groups of 5 animals, therefore of the 19 degrees of freedom, 3 correspond to diets, leaving 16 degrees of freedom on each day referring to the individual variation factor within diets. Thus on all 4 days we have 64 degrees of freedom coming from the individual variation factor within diets (if all had been on the same diet). There are also 12 degrees of freedom for the effect of the diets and 3 for the effect of the days, making up the total of 79 degrees of freedom. The variance of 64 degrees of freedom due to the individual variation factor may then be compared with that of 12 degrees of freedom due to diets.

To calculate variances. In one day there are 19 degrees of freedom, 16 due to individuals and 3 due to diets.

Let the mean of the results from all animals on that day = $\bar{x}$.

Let each observation = x.

and the mean of 5 observations in each heft = $\bar{x}_p$.

$x - \bar{x}_p$ = value for each animal in a heft - the mean for that heft.

A /

A table is then drawn up as follows:-

x	$\bar{x}_p$.	$x-\bar{x}$	$(x-\bar{x})^2$	$(x-\bar{x}_p)^2$	$\bar{x}_p-\bar{x}$	$(\bar{x}_p-\bar{x})^2$
			sum.	sum.		sum.

$5S(\bar{x}_p-\bar{x})^2 = Z$ = variance corresponding to 3 degrees of freedom due to diet.

$S(x-\bar{x})^2 = X$ = " " " 19 " " " due to individual variation factors and diet.

$S(x-\bar{x}_p)^2 = Y$ = " " " 16 degrees of freedom due to individual variation factor.

It may be verified that $X - Y = Z$.

For each day $\frac{Y}{16} = b$ = average variance per degree of freedom for individual variation factor.

$\frac{Z}{3} = a$ = average variance per degree of freedom for diet factor.

To find whether the two values a and b are significantly different or not we calculate $z = \frac{1}{2}(\log_e a - \log_e b)$.

Values z_1, z_2, z_3, and z_4 are calculated for the four days with corresponding values of Y and Z.

$Y_1 + Y_2 + Y_3 + Y_4 = P$ = total variance for individual variation factor on four days corresponding to 64 degrees of freedom.

Similarly $Z_1 + Z_2 + Z_3 + Z_4 = Q$ = total variance for diet factor over all days corresponding to 12 degrees of freedom.

Then $\frac{P}{64} = B$ = average variance per degree of freedom for individual variation factor over 4 days.

and $\frac{Q}{12} = A$ = average variance per degree of freedom for diet factor over 4 days.

To estimate the possible significance of the difference between A. and B. we calculate the observed value of $z = \frac{1}{2}(\log_e A - \log_e B)$. From Fisher's table we find the value of P corresponding to z, the table being entered with the appropriate//

appropriate values of n_1, n_2 the degrees of freedom relating to the two factors respectively . P as usual gives the probability that a discrepancy between A and B as great as or greater than that actually found would be expected as the result of chance. In the Appendix a corresponding numerical example will be found.

Some preliminary calculations with this method indicated the likelihood that considerable sections of the data would fail to show differences statistic-:ally significant and the desirability was evident of some simple means of discovering the direction in which statistically significant differences were most likely to be found, in order to curtail calculation. To this end the following simple test was applied. A particular reaction was selected and each day was considered separately. As explained above the twenty animals on that day can be divided into four groups, each on a different diet. The highest and lowest readings for single animals within a particular heft were taken and the difference between them was called the range. The mean of these ranges for each of the four separate hefts was then calculated. Secondly the mean reading for each heft was found and the range of these means i.e. the difference between the highest and the lowest was calculated. The ratio $\frac{\text{range of means}}{\text{mean of ranges}}$ formed a rough indication of the significance of the diet factor for that particular day. These ratios, then, for all the days in all the periods were arranged in descending order of magnitude and the analysis of variance method was then applied, beginning with the day showing the highest ratio. The results are shown in table XXIX.

As was expected the significance of the effect of diet on the various days as worked out by the analysis of variance method ran roughly parallel with the magnitude of the ratio worked out by the method given above and so it was only/

only necessary to apply the more laborious calculation to a number of days showing the highest ratio. It should be emphasised that no statistical

Table XXIX.

Haemolytic Reaction.		Titration of Complement.		Bactericidal reaction with B. suipestifer.		Agglutination reaction with B. abortus.	
Ratio.	P.	Ratio.	P.	Ratio.	P.	Ratio.	P.
1.50	<0.01	1.26	<0.01	1.2	>0.05	1.9	0.01-0.05
0.96		0.88	0.01-0.05	1.0	0.01-0.05	1.06	<0.01
0.94	0.01-0.05	0.74	insig.	0.8	insig.	0.93	0.01-0.05
0.92	0.01-0.05					0.8	insig.
0.88	0.01-0.05						
0.78	insig.						

The highest ratios for the bactericidal reaction with B.coli "X" were 1.03 and 0.8 and for the agglutination reaction with B. paratyphosus B. were 0.9 and 0.68. The corresponding values of P. were all insignificant.

importance is attached to these ratios in themselves, but they have been employed only as a means of avoiding a large amount of laborious calculation likely to lead to no positive results. That they should be of use in this respect depends on the fact that the numbers of animals employed and the division of the animals according to diet on practically all days were identic-:al. The few days in which this did not hold were not treated by the ratio method, but the method of analysis of variance was applied directly.

As a result of this process it was found, as had been anticipated that in the case of a number of reactions no effect even approaching significance of the variation in diet could be detected on any particular day. As in any month there were most only eight days it seemed safe to conclude that no substantially significant effect of the variations in diet would be found even

if/

if the eight days were taken together. This was borne out by more complete analysis of the variance of all the days in one period, which was made in one or two instances.

It may be mentioned at this point that variance analyses applied in a few instances showed that the day to day variation was of quite marked significance and could not be explained in terms of sampling variation, that is to say as due to the fact that different groups of animals were examined on different days. The results of such an analysis for January and March, 1931 are shown in the accompanying table.

Table XXX.

Values of P obtained on comparison of the different days within a sampling period.

Haemolytic Reaction.

March, 1931.	$P = < 0.01$
January, 1931.	$P = < 0.01$

Titration of Complement.

July, 1931.	P insignificant
November, 1931	$P = < 0.01$

In the case of the haemolysin results for March, the analysis pointed to the reality in this case of the effects of diet variation and so these figures were examined in greater detail. It should be pointed out here, that it happened that in this particular month the general scheme of the experiment was not strictly adhered to on two out of four days. On one, ten animals from each of two diet groups were examined, whilst on the other ten animals from each of the remaining two groups were employed. However, the method/

method of the analysis of variance could, of course, be applied with suitable modifications and results were obtained as shown in table XXXI.

Table XXXI.

March, 1931.

Days.	Hefts Examined.	Value of P.			Order of Hefts.
1.	I, II, III, IV.	>0.05			III > I > II > IV.
2.	I, II, III, IV.	0.01-0.05	0.01-0.05	<0.01	III > I > II > IV.
3.	II, III.	<0.01	<0.01		III > II.
4.	I, IV.	<0.01			I > IV.

It will be seen that the effect of diet is significant on three out of four days and also for the set of observations as a whole. It was obviously desirable to ascertain the order in which the diets fell and to what extent that order was significant. The order was readily found to be III, I, II, IV in decreasing strength and this order was consistent not only with the results of the period as a whole but also with those of each particular day. The question now arises as to whether the difference between any two particular diets is statistically significant.

This problem presents some difficulty as there is a day to day variation the effects of which must be allowed for in comparing the diets taken over the three or four days. The following method was used in which the difference of the means of the animals on two selected diets is compared, by the use of the "t" method of Fisher, but in calculating the variance of this difference allowance is made for the variance due to the days.

Let us suppose we are comparing diets I and II, and let a_1, b_1, c_1, and

d_1 /

d_1 be the means of the groups of animals on diet I and a_2, b_2, c_2 and d_2 the means of the animals on diet II on the four days respectively. Let $\bar{a}$, $\bar{b}$, $\bar{c}$ and $\bar{d}$ be the means of all the four diets on each of the four days. Then $a_1-\bar{a}$, $b_1-\bar{b}$, $c_1-\bar{c}$, and $d_1-\bar{d}$ may be taken as representing the effects of diet I on those four days, while $a_2-\bar{a}$, $b_2-\bar{b}$, $c_2-\bar{c}$ and $d_2-\bar{d}$ will represent the effect of diet II. We then look upon these two sets of four readings as two small samples and determine by the "t" method of Fisher whether they may be regarded as derived from a homogeneous population, as they would be if the diet were without effect. In other words, the mean of the four values $a_1-\bar{a}$, $b_1-\bar{b}$, $c_1-\bar{c}$ and $d_1-\bar{d}$ is compared for significance with the mean of $a_2-\bar{a}$, $b_2-\bar{b}$, $c_2-\bar{c}$ and $d_2-\bar{d}$. Strictly speaking, it might be desirable when the groups are not all of one size, as in the case of the March figures, to use some kind of weighting factor, but this complication did not seem necessary and it appears entirely improbable that any correction of this kind would substantially alter the result. When this method was applied the results shown in the following table were obtained:-

Table XXXII.

Hefts Compared.	Value of P.
I and II.	≤ 0.05
I and III.	>0.05
I and IV.	0.01
II and III.	<0.01
II and IV.	>0.05
III and IV.	<0.01

It will be seen that hefts III and I are significantly higher than IV and that III is higher than II. In other words, the order found above appears to have real significance but should not be pressed too far in detail, because, though

the/

the difference between the extreme members is quite statistically significant each group does not differ with statistical significance from its immediate neighbour.

The figures for the haemolytic reaction were next investigated in order to ascertain whether there were any seasonal variations and if so, the relationship of this to diet. For this purpose a particular diet group was taken in two months, e.g. October and January. In October we have the mean for the three groups in this diet, read on three successive days and in January a similar group of three readings. The means of these two sets of observations were then compared for significance by the "t" method. Clearly, if there were any seasonal variations the difference of the means, if any, should be entirely explicable in terms of the day to day variation. Strictly speaking, as the animal groups on successive days in the same month always consist of different animals, the groups in the second month ought to be composed of new animals also and not of those examined on the first occasion. A little consideration, however, will show that as the same animals are tested in each of the two seasons the means, if anything, will tend to agree better than when entirely different animals are examined on the second occasion. It follows that the P values given in Table XXXIII are rather greater than they ought to be and that the significances where they exist are probably underrated. The nature of the factors, however, does not seem to justify more complicated and laborious calculation.

Table/

Table XXXIII.

Values of P obtained on comparing various periods of sampling.

Haemolytic Reaction - Ewes.

Heft.	Oct-Jan. Fall.	Jan-Mar. Fall except heft III.	Oct-Mar. Fall.	Mar-May. Rise.	May-July.
I.	0.2	0.7	0.2	0.01	0.9
II.	0.05	1.0	0.02	<0.01	0.3
III.	0.2	0.2	0.5	0.1	0.7
IV.	0.02	0.7	<0.01	<0.01	0.3
I-IV.	0.5	0.7		0.02	>0.9

It will be seen from this table that the animals on diet III do not show any very significant change during the period of observation. On the other hand, diet IV shows a quite significant fall from October through January to March, followed by a significant rise from March to May. The animals on diet II behaved very similarly to those on diet IV, while in the case of diet I the fall is not very marked though the rise is significant. In other words, the animals on the basal diet showed a definite and significant fall in the haemolytic activity of their sera during the winter season followed by a rise during the spring. When the complete supplement was given in addition to the basal diet this variation was reduced in extent and indeed to such a degree that in these experiments its significance was no longer apparent. The other two diets gave intermediate results, II being almost the same as IV and I more like III. It is now seen how it was in March that the order came to be III, I, II, IV. The consistency of the results arrived at from the two points of view may be taken as evidence of their reality.

The above findings were obtained from the figures for ewes' sera, but similar methods applied to the results of the various reactions with the sera of/

of hoggs, gimmers, and barren ewes have failed to elicit any tendency towards significance, except on occasional days when it was probably due to chance. The hoggs, however, showed a rise in haemolytic activity of their sera from October to January, coincident with the fall occurring in the ewes' sera and this was found to be statistically significant when examined by a method similar to that employed in the case of the ewes (p. 97). With the hoggs, however, each heft was examined on only two days in each period so that the data is even more limited.

Table XXXIV.

Values of P obtained on comparing various periods of sampling.

Haemolytic Reaction - Hoggs.

Heft.	Oct-Jan.	Jan-Mar.	Mar-May.
I.	0.01-0.05	0.4	0.5-0.4
II.	0.02	0.6-0.5	0.7-0.6
III.	0.02	0.7-0.6	0.4
IV.	0.05-0.02	>0.9	0.5-0.4

SECTION VI.

LAMB DYSENTERY TOXIN TESTS.

The author took part in the work described in a recently published paper (Mackie, Fraser, Finkelstein and Anderson, 1932). This consisted of intracut-:aneous toxin tests carried out on sheep on the Garrochoran farm during 1931. After the clinical differences had been elicited it was considered desirable to test, in some manner more direct than the serological methods already discussed, the susceptibility to disease of the sheep in the various hefts. As direct infection was found inpracticable, it was decided to use the toxin tests referred to.

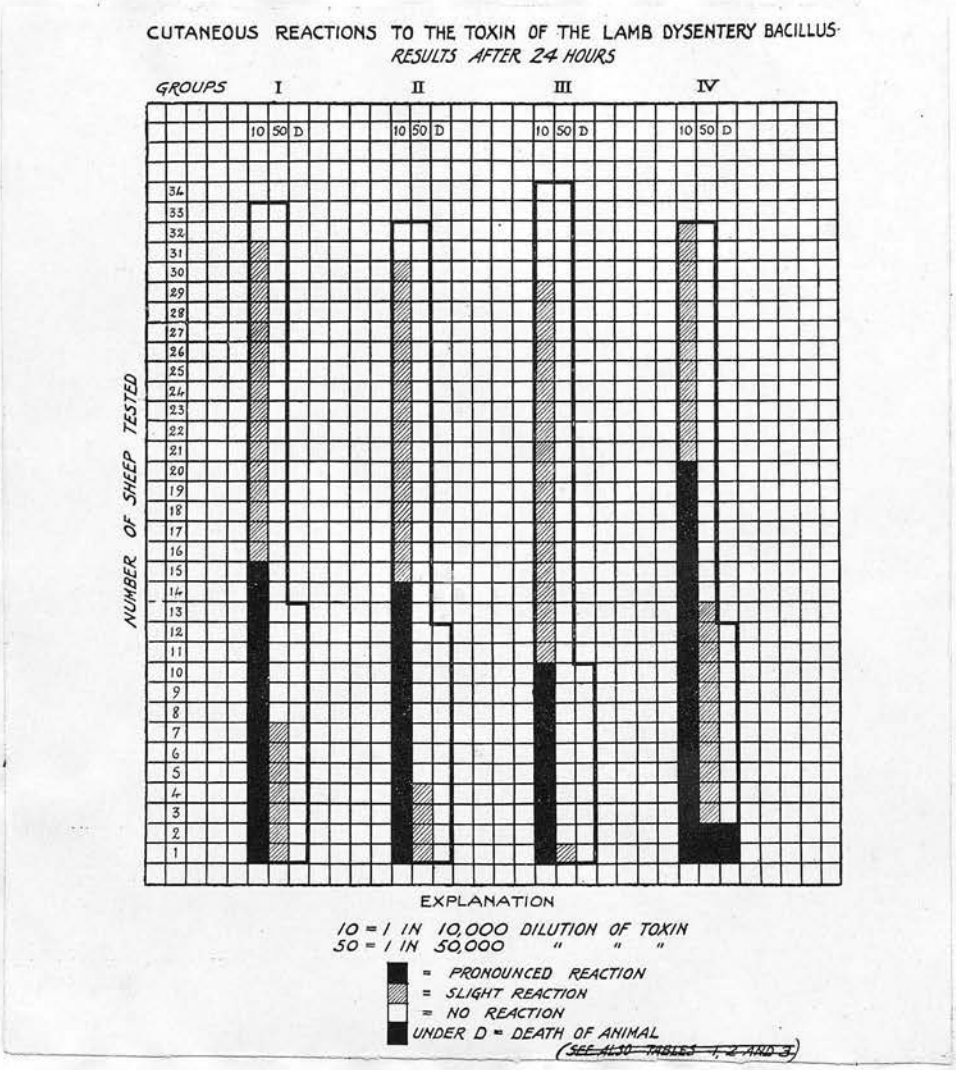
The lamb dysentery toxin used was the exotoxin of an organism closely allied to B. welchii (Dalling 1928). Glenny, Llewellyn Jones and Mason(1931) refer to the cutaneous reaction produced on infection of this toxin in laborat-:ory animals and quote earlier work.

Method.

A dried preparation of the toxin was obtained by courtesy of the Director of the Wellcome Research Laboratories and preliminary tests were carried out to find the most suitable dilutions for use. (It has been found necessary to repeat this before every series of tests, as the toxin tends to decrease in strength). During the original tests in May and June, 1931 the dilutions used were 1 : 1000, 1 : 10,000 and 1 : 50,000. The amount injected was 0.5 c.c. and the injection was made in the skin of the tail. The results were observed after 24 and 48 hours. It was found that 0.5 c.c. of a 1 : 1000 dilution/

Fig 27.

(Mackie, Fraser, Finkelstein and Anderson 1932)



dilution injected intracutaneously in sheep produced a marked reaction consist-:ing of a swollen purplish area associated with a varying amount of surrounding oedema.

A preliminary test in May with three ewe hoggs from each heft elicited striking differences. Those from heft IV gave definite reactions with the 1 : 50,000 dilution and one of them died. The animals from heft I showed marked reactions with the 1 :10,000 dilution and one of them gave a slight reaction with the 1 : 50,000 dilution. On the other hand, the animals from hefts II and III showed little or no reaction with the 1 : 10,000 dilution and only one of them gave a slight reaction with the 1 : 50,000 dilution.

Early in June a further series of tests were made on 18 - 20 animals of varying ages from each heft. In order to reduce the possibility of fatalities only a few animals were given the dilution 1 : 1000. As judged by the number of animals reacting to the 1 : 50,000 dilution of toxin, the susceptibility of the various hefts was in the order IV > I > II > III. Later in the same month further tests were carried out on groups of animals from the various hefts, comparable as regards age. By this time, however, an abundant supply of fresh grass was available for all hefts and the differences between the hefts almost entirely disappeared.

Figure 27 shows graphically the results of all tests carried out including the latter ones, which tend to make the differentiation less, but still leave it well marked.

The statistical significance of these differences was tested by the ($P\chi^2$) method/

method (described on page 33) which showed that the order of susceptibility was IV > I > II > III. The values of P calculated for these tests are given in the Appendix. There was no apparent relationship between susceptibility and age. Further tests were made in October but, as at the end of June, no differences were elicited.

SECTION VII.

DISCUSSION AND GENERAL CONCLUSION.

In the foregoing parts of this thesis the results of the work done have been considered from two different standpoints, first as they appeared to the observer without statistical analysis and secondly after critical statistical methods had been applied to them. Some of the differences which appeared on first inspection to be significant were not confirmed by the later statistical analysis. It has been shown that among sheep there are individual variations of considerable magnitude as well as day-to-day variations depending on unknown factors beyond experimental control. In fact, many of the differences which at first sight appeared to be correlated with diet or season were found to be explicable as due to the random operation of these individual and day-to-day variations. The aim of the statistical analysis has, therefore, been to allow for these two factors and to discover the differences due to other causes. The discussion is, therefore, limited to those results which have been found to be "statistically significant".

"Statistically significant" has been defined to mean that a result equally or more discrepant would be expected to occur less than once in twenty times as the result of chance. It is, therefore, to be expected that a result of this type would appear occasionally in the course of a large series of observa-:tions even although there was no real heterogeneity. In consequence it is desirable not to lay too much stress on borderline cases especially if they seem to be merely isolated occurrences. On the other hand, where a number of observations each of which is statistically significant, appear to converge

to/

to a general conclusion, such a conclusion may be put forward with more confidence. It may be added that, of course, even an isolated result with a P as low as, say, 0.001, would be given considerable weight but it will be found that values of P as low as this have been encountered only occasionally in the present investigation.

The facts elicited during this work regarding the effect of diet on the natural immunity reaction of serum have been of limited extent. Under the experimental conditions of the Ashtown experiment nothing of statistical significance was observed, until some of the animals were put out to grass towards the end of the experiment. In the course of the Garrochoran experiment, however, some significant differences were observed which may be attributed to diet. In each reaction a few significant differences appeared between hefts on widely separated days; but, as explained above, these were on the whole due to chance. In March, however, when the results of the haemolytic reaction of ewes' sera with rabbit erythrocytes were examined it was found that on each day of the period differences which were significant or nearly so were obtained and that significant differences appeared when the whole period was considered. The order of the hefts according to the haemolysin content of the sera was III >I >II >IV, i.e. the heft receiving the complete supplement was highest, that on the basal ration was lowest, while of the two intermediate groups that receiving additional calories was higher than that receiving a mineral supplement. It was shown that the sera from heft III had a significantly higher content than those from heft IV. In the case of the intracutaneous tests with lamb dysentery toxin in May and the beginning of June, the order of the hefts was III > II > I > IV, hefts III and II each being significantly different from IV.

The/

In each of these reactions the group receiving the complete supplement gave significantly higher results than that receiving only the basal ration, though it appears that the difference may have been due to different components of the supplement in the two cases. The difference between the results from the various hefts disappeared by May in the case of the haemolytic reaction and by the end of June in the case of the toxin tests. It is probable that this was in some measure at least due to the fact that by May fresh grass was available for all hefts in abundance. The order of the hefts as evidenced by the haemolytic reaction in March has been shown to arise from the different behaviour of the hefts from month to month. In the interval between the October and January samplings a fall in the haemolytic activity of the sera of all animals occurred, significant in hefts II and IV. By March a further slight fall appeared in hefts I and IV while heft II remained steady and heft III rose slightly. When the periods October and March were considered, it was seen that the decrease in haemolysin content of hefts II and IV was of statistical significance, while that in hefts I and III was not so. From March to May an increase in haemolytic power took place in all hefts, but was not significant in heft III. Thus, it appears that the supplementary feeding supplied to heft III, and in a lesser degree that supplied to heft I, was capable of preventing the fall in lytic action which occurred throughout the winter in the other animals not receiving these rations. It seems also that when fresh grass became available an increase in lytic power followed in hefts I, II and IV, but not in heft III which received the complete supplement. This suggests that some element was supplied by the complete supplement which was necessary for the maintenance of the level of this reaction in the serum

and/

and that grass also supplied that element.

Another explanation of the diminution of the haemolytic property of the ewes' sera comes to light, however, when the behaviour of the hoggs in this reaction from month to month is considered. In their case an increase occurred between October and January which has been shown to be statistically signific-:ant. It may thus be that the diminution in the case of ewes was due to pregnancy and that the increase in the spring occurred after parturition and was not entirely due to the effect of grass.

The climatic factor must also be taken into consideration as during the milder winter of 1931-32 this reduction of haemolytic activity of sheep serum did not take place in a manner sufficiently marked to make the results significant.

On the available evidence it may be concluded that when various unfavour-:able circumstances are acting simultaneously - e.g. adverse weather, inadequate diet and pregnancy - a significant fall in the haemolytic activity does actually take place in the sheep's blood serum, but supplementary feeding appears to prevent the development of this deficiency.

It should be emphasised again that both immune body and serum complement take part in the haemolytic reaction discussed here and that where the term haemolysin appears throughout the paper it has been used loosely to cover both these components. In these and the bactericidal reactions also no attempt has been made so far to find whether the variations occurring are due to changes in the serum content of complement or the immune body concerned.

If/

If the deficiency referred to above may be prevented by an entirely adequate diet, it would be expected that grass, the natural food of sheep, would be capable of removing it. It has already been stated that with the appearance of grass in May the deficiency tended to disappear, but too much emphasis cannot be laid on this, as the pregnancy factor also disappeared about the same time. However, it is interesting to note that in the Ashtown experiment, animals which were put out to grass when compared with control animals remaining under experimental conditions showed a significantly higher level in serum haemolysis as well as in the bactericidal reactions with B. coli "X" and B. suipestifer. On the other hand, the agglutination titre to B. abortus (Hog) of the former animals was significantly lower than that of the latter animals.

It may be convenient at this point to note another series of significant results which have been observed in the Ashtown experiments, namely, the differences between the animals in the indoor and outdoor groups taken as a whole without reference to the dietary groups. Here the indoor groups showed significantly higher results than the outdoor ones in March in the haemolytic reaction with rabbit erythrocytes and the bactericidal reaction with B.coli "X" but in the case of the agglutination reaction with B. abortus in this month the indoor animals gave on the average a lower titre than those outdoor. It is curious that the anomalous effect with B. abortus agglutination is also observed with respect to grass, as mentioned above. In March the bactericidal reaction with B. suipestifer showed no significant difference between the two groups, indoor and outdoor; but later in the year a difference developed, the indoor/

indoor titres becoming lower than the outdoor ones, which varied very little. These observations bring out the effect of "climate", a term under which influences such as ultra violet light may be included. The fact that the same effect is observed in the case of the animals which had to endure the severe winter at Garrochoran as in the case of the outdoor animals at Ashtown may indicate that irradiation is not so important as the other climatic factors such as wind, rain and temperature, and no doubt in the case of animals exposed to severe weather a higher number of calories would be demand-:ed than with animals not so exposed. The diets of high calorific value in the Garrochoran experiment, namely, those of hefts I and III, have already been shown in the case of haemolytic reaction to be the most effective, so that it may be inferred that the factor chiefly responsible for low haemolysin content and other abnormal reactions is probably a deficiency in the calorific value of the foodstuffs relative to the environment of the animal. The effect of pregnancy would also be readily understood on this view.

The differences noted between results obtained from the sheep at Ashtown and those at Garrochoran may be remarked on. It was considered that this might be due to difference in breed, the former being Scottish half-bred stock and the latter Scottish blackfaced sheep. The figures obtained at the first sampling at Garrochoran when compared with results from similar sheep of the same breed at Aberdeen were significantly lower in the case of the haemolytic reaction, and the agglutination reactions with B. paratyphosus B. and B. abortus (Hog). In the case of the bactericidal reaction with B.coli"X" the differences were indeterminate. The results from the black-faced sheep at Aberdeen were then compared with those from sheep as nearly as possible

similar/

similar in all respects other than breed, these being of the Border-Leicester stock. Here it was found that a significant difference was present only in the haemolytic reaction. It thus appears that while breed may have some slight influence, this effect is not anything like so great as the influence exerted by environmental factors or dietary conditions.

Hoggs and ewes wintered at Aberdeen in 1930-31 were also compared with similar animals sampled at the same periods at Garrochoran. The ewes at Aberdeen showed a higher haemolytic activity at all periods but no consistent differences in the other reactions. The hoggs at Aberdeen exhibited a higher bactericidal power towards B. suipestifer at all times of sampling, which disappeared after their return to Garrochoran.

The work recorded in the thesis, as explained earlier, was essentially of an exploratory nature and was undertaken initially to ascertain whether the natural reactions of the blood serum and the activity of the underlying immunity mechanism were influenced by dietary factors. The observations made have revealed the great complexity of the subject and have shown how in nature these physiological properties are influenced by other factors which must be allowed for in any nutritional study of this kind. Many of the results have been entirely negative as might be expected in an exploratory study but the inquiry has served its original purpose namely to "clear the ground" and provide pointers for further and more specific investigations.

The statistical part of the investigation has been of particular interest as an application of statistical methods to a biological inquiry involving large scale "sampling" from individual animals. It has shown how variations/

variations due to different factors may be critically analysed.

As regards the essential question whether resistance to infective disease is altered by dietary factors, among the various observations recorded the tests of susceptibility to a bacterial toxin have provided perhaps the most acceptable evidence that immunity is influenced by nutrition but it is still of special interest that a correlation can also be shown in differently dieted groups of animals between the results of such an in vivo reaction and an in vitro serum reaction dependent on a natural antibody acting along with complement, at least in so far as groups on basal ration and basal ration + complete supplement of minerals, cod liver oil and maize are concerned.

The effect of natural grass feeding, the difference between the winter and the summer grazing, and the possible effect of the calorific value of the diet, have been specially interesting results of the investigation as a whole.

The inverse ratio of the lytic and agglutination reactions is particularly noteworthy, though it is difficult to offer any explanation of this peculiarity at the present stage of the work.

In conclusion,it must be clearly recognised that, while evidence has been elicited that dietary deficiencies affect the natural immunity mechanism, at the present stage no statement can be made regarding the relative importance of individual dietary factors. The work done, however, constitutes a step towards further study of this important question.

These experiments in sheep, however, require to be followed up now by observations in small animals in which susceptibility to infection can be tested by direct methods.

ACKNOWLEDGEMENTS.

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To the other members of the team engaged in this research - Dr.A.H.H. Fraser, Dr. W.A. Carr-Fraser, Dr. D.W. Auchinachie, Mr C.A. Puddy and Mr G. Jamieson - my thanks are due for assistance in the sampling and transport of the blood.

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A preliminary note on this investigation was published in 1931 (Orr, Macleod, Mackie.)

BIBLIOGRAPHY.

Arkwright, J. A. & Zilva, S.S. (1924) J. Path. & Bact. , 27, 346.

Blake, A.V. & Okell, C.C. (1929) Brit. J.Exper. Path. , 10, 175.

Cramer, W. & Kingsbury, A.N. (1924) Brit. J. Exper. Path, 5, 300.

Dalling, F. (1928) Vet. Rec., 8, 841.

Findlay, G. M. (1923) J. Path. & Bact., 26, 1,485.

Fisher, R.A. (1925) Statistical Methods for Research Workers. (Oliver & Boyd).

Gibson, H. J. (1930) J. Hyg., 30, 337.

Glenny, A.T.,Llewellyn-Jones,M., & Mason, J.H. (1931) J.Path. & Bact.,34,201.

Godden, W. (1926) J. Agric. Sci., 16, 78.

Kraus, R. & Clairmont, P. (1900) Z. Hyg. Infektkr. 34, 1.

Lassen, H.C.A. (1930) J. Hyg., 30, 301.

Lawrynowicz, M.A. (1931) Jour. de Phys. et de Path. Gen. 29,(2),270.

Ledingham, J.C.G. (1931) A.System of Bacteriology in relation to Medicine, 6, 49. (Med. Res. Counc.)

Mackie, T. J. & Finkelstein,M.H. (1930) J. Hyg., 30, 1.
(1931) ibid. 31, 35.
(1932) ibid. 32, 1

Mackie, T. J., Fraser, A.H.H., Finkelstein, M.H. & Anderson, E.J.M. (1932) Brit. J. Exper. Path. 13, 328.

Mellanby, E. & Green, H.N. (1929) Brit. Med. J., 1, 984.

Orr, J. B. & Fraser, A. H. H.(1932) Trans. Highland & Agric. Soc. Scotland, 44, 64.

Orr, J. B., Macleod, J.J.R. & Mackie, T.J. (1931) Lancet, Vol. I, No.5622,May.

Osborn, T.W.B. (1931) Biochem. J., 25, 2136.

Reid. (1916) Scottish Farmer, 8th Jan.

Smith/

Smith, G.H. & Wason, J.M. (1923) J. Immunol. 8, 195.

Topley, W.W.C., Greenwood, M. & Wilson, J. (1931) J. Path & Bact., 34, 163.

Tromsdorff, R. (1902) Zbl. Bakt. Orig. 32, 439.

Tunnicliff, R. (1931) J. Infect. Dis., 48, 161.

Vasile, B. (1929) Pediatria, 37, 1059.

Webster, L.T. & Pritchett, I.W. (1924) J. Exper. Med., 40, 397.

Weiss, St., Sümegi, St. & Benkovico, Z. (1928) Ztschr. Ges. Exp. Med., 60, 697.

Yule, G. U. (1922) J. Roy. Stat. Soc., 85, Part I, 95.

Zilva, S.S. (1919) Biochem. J., 13, 172.

A P P E N D I X.

PROTOCOLS

OF

ASHTOWN EXPERIMENT.

Results of Haemolytic Reaction are given in M.H.D.s per c.c.

" " Bactericidal Reactions are given in Bactericidal Units.

" " Agglutination Reactions are given in Agglutination Units.

ERRATUM.

The results of the agglutination tests have been expressed in the protocols of the Ashtown and Garrochoran Experiments, not, as stated, in agglutination units, but as the denominator of the highest dilution of serum in which agglutination occurred, the numerator being 1 in each case.

ASHTOWN EXPERIMENT 1929-30.

GROUP I - INDOOR.

	Number.	178.	112.	146.	153.	150.
Dec. 1929.	Haemolytic Reaction.	-	-	-	3.3	3.3
	Bactericidal reaction with B.suipestifer	8	8	6	9	6
	Bactericidal reaction with B.coli "X"	-	-	-	-	-
	Agglutination reaction with B.paratyphosus B.	16	16	16	16	-
	Agglutination reaction with B.abortus (Hog)	-	-	-	-	128
Jan. 1930.	Haemolytic Reaction.	10.0	5.0	13.3	5.0	4.0
	Bactericidal reaction with B.suipestifer	4	5	5	4	4
	Bactericidal reaction with B.coli "X"	0	2	0	0	0
	Agglutination reaction with B.paratyphosus B.	64	128	128	32	32
	Agglutination reaction with B.abortus (Hog)	32	16	64	64	64
Mar.	Haemolytic Reaction.	10.0	6.6	10.0	6.6	6.6
	Bactericidal reaction with B.suipestifer	4	2	5	4	4
	Bactericidal reaction with B.coli "X"	2	2	0	0	2
	Agglutination reaction with B.paratyphosus B.	16	32	16	64	64
	Agglutination reaction with B.abortus (Hog)	128	64	128	256	64
May.	Haemolytic Reaction.	4.0	5.0	4.0	5.0	5.0
	Bactericidal reaction with B.suipestifer	2	-	4	4	4
	Bactericidal reaction with B.coli "X"	0	2	0	0	0
	Bactericidal reaction with B. coli "F_3"	0	0	0	0	0
	Agglutination reaction with B.paratyphosus B.	32	16	16	32	16
	Agglutination reaction with B.abortus (Hog)	128	32	-	128	-
Sept.	Haemolytic Reaction *	2.8	-	4.0 *	2.8 *	4.0 *
	Bactericidal reaction with B.suipestifer	5	-	4	4	5
	Bactericidal reaction with B.coli "X"	2	-	2	2	2
	Bactericidal reaction with B. coli "F_3"	0	-	2	0	0
	Agglutination reaction with B.paratyphosus B.	128	-	64	32	64
	Agglutination reaction with B.abortus (Hog)	64	-	32	64	32

ASHTOWN EXPERIMENT 1929-30.

GROUP II - INDOOR.

	Number.	144.	155.	166.	125.	175.
Dec.	Haemolytic Reaction.	4.0	5.0	5.0	2.8	-
1929.	Bactericidal (B. suipestifer	8	9	10	6	-
	reaction with(B. coli "X"	-	-	-	-	-
	Agglutination(B.paratyphosus B.	16	32	8	-	32
	reaction with(B.abortus (Hog)	-	-	-	64	-
Jan.	Haemolytic Reaction.	10.0	6.6	10.0	5.0	5.0
1930.	Bactericidal (B.suipestifer	4	4	4	5	4
	reaction with(B.coli "X"	0	0	0	6	0
	Agglutination(B. paratyphosus B.	16	32	128	64	64
	reaction with(B.abortus (Hog)	64	64	128	32	32
Mar.	Haemolytic Reaction.	10.0	6.6	6.6	10.0	6.6
	Bactericidal (B. suipestifer	4	4	5	5	4
	reaction with(B. coli "X"	2	0	0	2	2
	Agglutination(B.paratyphosus B.	64	128	8	32	128
	reaction with(B.abortus (Hog)	128	256	64	64	256
May.	Haemolytic Reaction.	3.3	5.0	4.0	5.0	4.0
	Bactericidal (B.suipestifer	2	-	4	2	-
	reaction with(B.coli "X"	0	0	0	0	0
	(B. coli "F_3"	0	0	0	0	0
	Agglutination(B. paratyphosus B.	32	32	32	32	32
	reaction with(B.abortus (Hog)	128	256	-	64	128
Sept.	Haemolytic Reaction.	2.2	2.8	4.0 *	6.6 *	2.8
	Bactericidal (B.suipestifer	2	3	7	6	3
	reaction with(B.coli "X"	0	0	0	2	0
	(B. coli "F_3)	0	0	2	0	0
	Agglutination(B.paratyphosus B.	64	16	32	16	32
	reaction with(B.abortus (Hog)	128	-	64	64	64

ASHTOWN EXPERIMENT 1929-30.

GROUP III - INDOOR.

	Number.	163.	119.	165.	123.	170.
Dec. 1929.	Haemolytic Reaction.	-	-	-	3.3	-
	Bactericidal reaction with *B. suipestifer*	6	8	10	8	9
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B*	32	16	16	16	8
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	-
Jan. 1930.	Haemolytic Reaction	6.6	13.3	5.0	5.0	5.0
	Bactericidal reaction with *B. suipestifer*	4	5	4	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	2
	Agglutination reaction with *B. paratyphosus B.*	64	128	32	32	128
	Agglutination reaction with *B. abortus* (Hog)	32	64	128	32	16
Mar.	Haemolytic Reaction	10.0	10.0	10.0	6.6	10.0
	Bactericidal reaction with *B. suipestifer*	4	4	4	5	4
	Bactericidal reaction with *B. coli* "X"	4	2	0	0	2
	Agglutination reaction with *B. paratyphosus B.*	64	64	8	32	32
	Agglutination reaction with *B. abortus* (Hog)	64	64	128	128	64
May.	Haemolytic Reaction.	5.0	5.0	5.0	5.0	5.0
	Bactericidal reaction with *B. suipestifer*	-	4	4	4	-
	Bactericidal reaction with *B. coli* "X"	2	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	2	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	8	32	32	16
	Agglutination reaction with *B. abortus* (Hog)	128	128	128	-	128
Sept.	Haemolytic Reaction.	* 4.0	* 4.0	* 4.0	4.0	4.0
	Bactericidal reaction with *B. suipestifer*	4	4	6	0	2
	Bactericidal reaction with *B.coli* "X"	2	2	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	2	0	0	0	0
	Agglutination reaction with *B. paratyphosus B*	4	64	32	16	16
	Agglutination reaction with *B. abortus* (Hog)	16	64	8	128	64

ASHTOWN EXPERIMENT 1929-30.

GROUP IV - INDOOR.

	Number.	117.	120.	182.	183.	191.
Dec.	Haemolytic Reaction.	4.0	-	3.3	-	-
1929.	Bactericidal (B. suipestifer	9	10	8	-	10
	reaction with(B. coli "X"	-	-	-	-	-
	Agglutination(B. paratyphosus B.	8	32	-	-	32
	reaction with(B. abortus (Hog)	-	-	64	-	-
Jan.	Haemolytic Reaction.	40.0	4.0	5.0	6.6	4.0
1930.	Bactericidal (B. suipestifer	4	4	4	5	4
	reaction with(B. coli "X"	0	2	6	0	0
	Agglutination(B. paratyphosus B.	128	64	128	64	64
	reaction with(B. abortus (Hog)	256	64	32	32	64
Mar.	Haemolytic Reaction.	10.0	5.0	6.6	10.0	6.6
	Bactericidal (B.suipestifer	5	4	4	5	4
	reaction with(B. coli "X"	0	4	2	6	0
	Agglutination(B. paratyphosus B.	16	64	128	128	32
	reaction with(B. abortus (Hog)	128	128	64	64	64
May.	Haemolytic Reaction.	5.0	5.0	3.3	4.0	5.0
	Bactericidal (B.suipestifer	4	-	2	2	-
	reaction with(B. coli "X"	0	2	0	0	0
	(B. coli "F_3"	0	0	0	0	0
	Agglutination(B. paratyphosus B.	32	16	8	16	16
	reaction with(B. abortus (Hog)	-	64	64	64	32
Sept.	Haemolytic Reaction.	2.8	4.0	4.0	6.6 *	4.0
	Bactericidal (B. suipestifer	3	3	2	5	2
	reaction with(B. coli "X"	0	0	0	2	0
	(B. coli "F_3"	0	0	0	0	0
	Agglutination(B. paratyphosus B.	64	32	64	64	128
	reaction with(B. abortus (Hog)	32	32	32	64	32

ASHTOWN EXPERIMENT 1929-30.

GROUP V - INDOOR.

	Number.	130.	156.	176.	116.	188.
Dec. 1929.	Haemolytic Reaction.	-	-	-	-	-
	Bactericidal reaction with *B. suipestifer*	8	-	10	10	-
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	16	16	16	-	-
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	-
Jan. 1930.	Haemolytic Reaction.	6.6	6.6	3.3	6.6	5.0
	Bactericidal reaction with *B. suipestifer*	5	4	5	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	2	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	32	64	32	64
	Agglutination reaction with *B. abortus* (Hog)	64	32	64	128	32
Mar.	Haemolytic Reaction.	10.0	10.0	10.0	5.0	6.6
	Bactericidal reaction with *B. suipestifer*	4	4	4	4	5
	Bactericidal reaction with *B. coli* "X"	2	2	2	2	0
	Agglutination reaction with *B. paratyphosus B.*	32	16	64	128	8
	Agglutination reaction with *B. abortus* (Hog).	128	256	64	256	128
May.	Haemolytic Reaction.	5.0	2.5	10.0	5.0	3.3
	Bactericidal reaction with *B. suipestifer*	2	2	-	4	-
	Bactericidal reaction with *B. coli* "X"	0	0	2	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	8	16	8	64
	Agglutination reaction with *B. abortus* (Hog)	256	64	128	-	128
Sept.	Haemolytic Reaction	-	2.8	4.0	-	2.8
	Bactericidal reaction with *B. suipestifer*	-	2	2	-	3
	Bactericidal reaction with *B. coli* "X"	-	0	0	-	0
	Bactericidal reaction with *B. coli* "F_3"	-	0	0	-	0
	Agglutination reaction with *B. paratyphosus B.*	-	256	128	-	64
	Agglutination reaction with *B. abortus* (Hog)	-	128	128	-	8

ASHTOWN EXPERIMENT 1929-30.

GROUP VI - INDOOR.

	Number.	132.	118.	187.	189.	160.
Dec. 1929.	Haemolytic Reaction.	-	-	5.0	2.8	-
	Bactericidal reaction with *B. suipestifer*	10	10	9	9	-
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	16	32	8	-	-
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	256	-
Jan. 1930.	Haemolytic Reaction.	6.6	5.0	10.0	5.0	5.0
	Bactericidal reaction with *B. suipestifer*	4.	5	4	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	64	32	32	32
	Agglutination reaction with *B. abortus* (Hog)	64	64	64	32	32
Mar.	Haemolytic Reaction.	5.0	10.0	6.6	10.0	10.0
	Bactericidal reaction with *B. suipestifer*	4	4	4	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	2	4
	Agglutination reaction with *B. paratyphosus B.*	64	16	128	16	16
	Agglutination reaction with *B. abortus* (Hog)	128	64	128	128	64
May.	Haemolytic Reaction.	5.0	4.0	3.3	3.3	3.3
	Bactericidal reaction with *B. suipestifer*	4	-	-	2	2
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	64	32	16	8
	Agglutination reaction with *B. abortus* (Hog)	-	128	512	128	64
Sept.	Haemolytic Reaction.	4.0	2.8	2.8	4.0	* 4.0
	Bactericidal reaction with *B. suipestifer*	2	2	3	3	5
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	2
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B*	128	64	64	128	64
	Agglutination reaction with *B. abortus* (Hog).	128	256	64	256	32

ASHTOWN EXPERIMENT 1929-30.

GROUP VII - INDOOR.

	Number.	157.	184.	190.	138.	177.
Dec. 1929.	Haemolytic Reaction.	5.0	3.3	-	-	-
	Bactericidal reaction with *B. suipestifer*	10	8	6	-	-
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	4	-	32	-	-
	Agglutination reaction with *B. abortus* (Hog)	-	256	-	-	-
Jan. 1930.	Haemolytic Reaction.	5.0	6.6	10.0	5.0	6.6
	Bactericidal reaction with *B. suipestifer*	4	4	4	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	4	0
	Agglutination reaction with *B. paratyphosus B.*	32	128	128	32	64
	Agglutination reaction with *B. abortus* (Hog)	64	32	256	16	64
Mar.	Haemolytic Reaction.	6.6	10.0	10.0	6.6	10.0
	Bactericidal reaction with *B. suipestifer*	4	4	5	4	4
	Bactericidal reaction with *B. coli* "X"	2	0	0	2	2
	Agglutination reaction with *B. paratyphosus B.*	32	32	16	32	64
	Agglutination reaction with *B. abortus* (Hog)	64	256	-	128	256
May.	Haemolytic Reaction.	5.0	5.0	4.0	4.0	4.0
	Bactericidal reaction with *B. suipestifer*	2	4	4	-	-
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	2
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	32	32	32	32
	Agglutination reaction with *B. abortus* (Hog)	64	-	64	64	128
Sept.	Haemolytic Reaction. *	5.0	2.8	4.0 *	4.0 *	4.0
	Bactericidal reaction with *B. suipestifer*	4	3	4	5	5
	Bactericidal reaction with *B. coli* "X"	2	2	0	0	2
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	2	0
	Agglutination reaction with *B. paratyphosus B.*	8	64	32	128	128
	Agglutination reaction with *B. abortus* (Hog)	32	128	64	64	64

ASHTOWN EXPERIMENT 1929-30.

GROUP VIII - INDOOR.

	Number.	151.	149.	162.	135.	179.
Dec. 1929.	Haemolytic Reaction.	-	5.0	4.0	4.0	3.3
	Bactericidal reaction with *B. suipestifer*	-	8	8	6	8
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	-	32	16	16	16
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	-
Jan. 1930.	Haemolytic Reaction.	10.0	10.0	6.6	6.6	10.0
	Bactericidal reaction with *B. suipestifer*	5	4	4	5	4
	Bactericidal reaction with *B. coli* "X"	0	2	0	2	2
	Agglutination reaction with *B. paratyphosus B.*	128	64	32	64	32
	Agglutination reaction with *B. abortus* (Hog)	64	32	32	32	64
Mar.	Haemolytic Reaction.	10.0	10.0	10.0	10.0	4.0
	Bactericidal reaction with *B. suipestifer*	4	4	4	2	4
	Bactericidal reaction with *B. coli* "X"	0	4	0	2	2
	Agglutination reaction with *B. paratyphosus B.*	8	16	64	32	64
	Agglutination reaction with *B. abortus* (Hog)	64	64	256	128	256
May.	Haemolytic Reaction.	4.0	5.0	6.6	4.0	4.0
	Bactericidal reaction with *B. suipestifer*	-	2	-	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	32	32	16	16
	Agglutination reaction with *B. abortus* (Hog)	64	64	64	-	-
Sept.	Haemolytic Reaction.	-	4.0	2.8	2.2 *	2.2
	Bactericidal reaction with *B. suipestifer*	-	2	2	5	5
	Bactericidal reaction with *B. coli* "X"	-	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	-	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	-	64	128	64	64
	Agglutination reaction with *B. abortus* (Hog).	-	64	128	32	32

ASHTOWN EXPERIMENT 1929-30.

GROUP I - OUTDOOR.

	Number.	186.	133.	154.	172.	174.
Dec. 1929.	Haemolytic Reaction.	-	4.0	3.3	-	4.0
	Bactericidal reaction with *B. suipestifer*	6	8	9	-	9
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	16	32	-	32	8
	Agglutination reaction with *B. abortus* (Hog)	-	-	64	-	-
Jan. 1930.	Haemolytic Reaction.	6.6	5.0	10.0	10.0	5.0
	Bactericidal reaction with *B. suipestifer*	4	4	4	4	6
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	2
	Agglutination reaction with *B. paratyphosus B.*	32	64	64	64	32
	Agglutination reaction with *B. abortus* (Hog)	64	64	128	64	64
Mar.	Haemolytic Reaction.	5.0	6.6	10.0	6.6	5.0
	Bactericidal reaction with *B. suipestifer*	5	4	5	4	4
	Bactericidal reaction with *B. coli* "X"	2	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	16	32	32	32
	Agglutination reaction with *B. abortus* (Hog)	256	128	128	128	128
May.	Haemolytic Reaction.	5.0	5.0	5.0	4.0	4.0
	Bactericidal reaction with *B. suipestifer*	4	-	4	4	-
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	128	4	32	64	32
	Agglutination reaction with *B. abortus* (Hog)	64	32	64	-	32
Sept.	Haemolytic Reaction. *	4.0	2.8	2.8	2.8	2.8
	Bactericidal reaction with *B. suipestifer*	5	3	2	3	3
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	64	64	64	16
	Agglutination reaction with *B. abortus* (Hog)	256	64	128	256	256

ASHTOWN EXPERIMENT 1929-30.

GROUP II - OUTDOOR.

	Number.	145.	141.	164.	113.	168.
Dec.	Haemolytic Reaction.	3.3	2.8	-	-	-
1929.	Bactericidal reaction with B.suipestifer	9	9	10	10	6
	Bactericidal reaction with B. coli "X"	-	-	-	-	-
	Agglutination reaction with B.paratyphosus B.	32	-	16	8	16
	Agglutination reaction with B.abortus (Hog)	-	64	-	-	-
Jan.	Haemolytic Reaction.	10.0	6.6	6.6	6.6	10.0
1930.	Bactericidal reaction with B.suipestifer	4	4	4	4	4
	Bactericidal reaction with B.coli "X"	0	0	0	0	0
	Agglutination reaction with B. paratyphosus B.	64	32	64	64	64
	Agglutination reaction with B.abortus (Hog)	64	64	64	256	32
Mar.	Haemolytic Reaction.	6.6	10.0	6.6	5.0	5.0
	Bactericidal reaction with B. suipestifer	4	4	4	4	5
	Bactericidal reaction with B.coli "X"	0	0	0	0	0
	Agglutination reaction with B.paratyphosus B.	16	16	32	16	32
	Agglutination reaction with B.abortus (Hog)	128	64	256	64	64
May.	Haemolytic Reaction.	4.0	5.0	5.0	3.3	5.0
	Bactericidal reaction with B.suipestifer	4	2	4	-	-
	Bactericidal reaction with B.coli "X"	0	2	2	0	2
	Bactericidal reaction with B.coli "F_3"	0	0	0	0	0
	Agglutination reaction with B.paratyphosus B.	8	16	32	32	32
	Agglutination reaction with B.abortus (Hog)	-	64	-	256	64
Sept.	Haemolytic Reaction.	5.0	4.0	4.0	-	4.0
	Bactericidal reaction with B.suipestifer	3	3	3	-	2
	Bactericidal reaction with B.coli "X"	0	0	0	-	0
	Bactericidal reaction with B.coli "F_3"	0	0	0	-	0
	Agglutination reaction with B.paratyphosus B.	32	16	32	-	64
	Agglutination reaction with B.abortus (Hog)	64	128	128	-	64

ASHTOWN EXPERIMENT 1929-30.

GROUP III - OUTDOOR.

	Number.	140.	121.	180.	137.	159.
Dec.	Haemolytic Reaction.	-	-	5.0	5.0	2.8
1929.	Bactericidal reaction with *B. suipestifer*	8	10	8	9	9
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	8	64	16	4	-
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	64
Jan.	Haemolytic Reaction.	10.0	6.6	10.0	10.0	4.0
1930.	Bactericidal reaction with *B. suipestifer*	4	5	4	4	6
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	2
	Agglutination reaction with *B. paratyphosus B.*	128	-	256	32	64
	Agglutination reaction with *B. abortus* (Hog)	64	32	32	64	128
Mar.	Haemolytic Reaction.	5.0	6.6	10.0	5.0	5.0
	Bactericidal reaction with *B. suipestifer*	4	4	4	4	5
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	32	32	32	32
	Agglutination reaction with *B. abortus* (Hog)	128	128	256	128	256
May.	Haemolytic Reaction.	4.0	5.0	5.0	6.6	4.0
	Bactericidal reaction with *B. suipestifer*	4	2	4	-	-
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	32	<4	32	64
	Agglutination reaction with *B. abortus* (Hog)	-	64	256	64	32
Sept.*	Haemolytic Reaction.	4.0	4.0	4.0	2.8	2.2
	Bactericidal reaction with *B. suipestifer*	5	3	3	4	2
	Bactericidal reaction with *B. coli* "X"	2	0	0	0	2
	Bactericidal reaction with *B. coli* "F_3"	2	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	128	64	16	32
	Agglutination reaction with *B. abortus* (Hog)	64	64	256	32	128

ASHTOWN EXPERIMENT 1929-30.

GROUP IV - OUTDOOR.

	Number.	147.	142.	152.	128.	126.
Dec. 1929.	Haemolytic Reaction.	-	4.0	-	3.3	-
	Bactericidal reaction with *B. suipestifer*	10	10	10	9	-
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	16	8	32	-	-
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	128	-
Jan. 1930.	Haemolytic Reaction.	10.0	10.0	10.0	5.0	6.6
	Bactericidal reaction with *B. suipestifer*	4	5	4	6	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	32	32	32	64
	Agglutination reaction with *B. abortus* (Hog)	128	32	128	32	256
Mar.	Haemolytic Reaction.	10.0	6.6	5.0	5.0	5.0
	Bactericidal reaction with *B. suipestifer*	4	5	4	2	4
	Bactericidal reaction with *B. coli* "X"	0	2	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	32	32	64	32
	Agglutination reaction with *B. abortus* (Hog)	64	128	256	64	128
May.	Haemolytic Reaction.	6.6	-	5.0	4.0	3.3
	Bactericidal reaction with *B. suipestifer*	-	-	-	2	4
	Bactericidal reaction with *B. coli* "X"	0	-	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	-	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	-	16	8	16
	Agglutination reaction with *B. abortus* (Hog)	64	-	64	128	128
Sept.	Haemolytic Reaction.	2.8	2.2	2.2 *	4.0	2.8
	Bactericidal reaction with *B. suipestifer*	5	4	5	5	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	2	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	128	128	32	64
	Agglutination reaction with *B. abortus* (Hog)	64	64	32	16	32

ASHTOWN EXPERIMENT 1929-30.

GROUP V - OUTDOOR.

	Number.	167.	139.	129.	114.	161.
Dec. 1929.	Haemolytic Reaction.	2.8	4.0	5.0	-	-
	Bactericidal reaction with *B. suipestifer*	8	8	10	8	8
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	-	16	8	16	16
	Agglutination reaction with *B. abortus* (Hog)	128	-	-	-	-
Jan. 1930.	Haemolytic Reaction.	6.6	6.6	6.6	6.6	5.0
	Bactericidal reaction with *B. suipestifer*	4	4	4	5	6
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	64	32	32	32
	Agglutination reaction with *B.abortus* (Hog)	64	128	256	32	32
Mar.	Haemolytic Reaction.	-	6.6	6.6	5.0	6.6
	Bactericidal reaction with *B. suipestifer*	-	4	4	5	4
	Bactericidal reaction with *B. coli* "X"	-	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	-	64	32	32	64
	Agglutination reaction with *B. abortus* (Hog)	-	128	256	256	256
May.	Haemolytic Reaction.	-	13.3	5.0	4.0	5.0
	Bactericidal reaction with *B. suipestifer*	-	-	-	4	4
	Bactericidal reaction with *B. coli* "X"	-	0	2	0	0
	Bactericidal reaction with *B. coli* "F_3"	-	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	-	-	8	32	64
	Agglutination reaction with *B. abortus* (Hog)	-	64	64	64	-
Sept.	Haemolytic Reaction.	-	2.8	5.0	2.8	2.8
	Bactericidal reaction with *B. suipestifer*	-	5	4	5	2
	Bactericidal reaction with *B. coli* "X"	-	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	-	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	-	128	32	64	64
	Agglutination reaction with *B. abortus* (Hog)	-	128	32	128	64

ASHTOWN EXPERIMENT 1929-30.

GROUP VI - OUTDOOR.

	Number.	185.	127.	143.	173.	134.
Dec. 1929.	Haemolytic Reaction.	-	-	-	-	-
	Bactericidal reaction with *B. suipestifer*	6	6	6	-	-
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	32	32	-	-	-
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	-
Jan. 1930.	Haemolytic Reaction.	5.0	5.0	10.0	6.6	5.0
	Bactericidal reaction with *B. suipestifer*	6	5	5	4	4
	Bactericidal reaction with *B. coli* "X"	2	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	64	64	64	64
	Agglutination reaction with *B. abortus* (Hog)	128	64	64	64	64
Mar.	Haemolytic Reaction.	6.6	5.0	10.0	5.0	6.6
	Bactericidal reaction with *B. suipestifer*	5	4	5	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	64	16	64	32
	Agglutination reaction with *B. abortus* (Hog)	128	128	128	128	64
May.	Haemolytic Reaction.	5.0	4.0	5.0	4.0	5.0
	Bactericidal reaction with *B. suipestifer*	4	-	-	4	4
	Bactericidal reaction with *B. coli* "X"	2	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	32	16	64	4
	Agglutination reaction with *B. abortus* (Hog)	128	32	64	-	64
Sept.	Haemolytic Reaction.	2.8	4.0 *	4.0	2.8	2.2
	Bactericidal reaction with *B. suipestifer*	5	5	4	4	4
	Bactericidal reaction with *B. coli* "X"	0	2	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	64	32	128	64	64
	Agglutination reaction with *B. abortus* (Hog)	16	32	64	32	32

ASHTOWN EXPERIMENT 1929-30.

GROUP VII - OUTDOOR.

	Number.	123.	158.	115.	169.	124.
Dec. 1929.	Haemolytic Reaction.	3.3	3.3	5.0	-	-
	Bactericidal reaction with *B. suipestifer*	8	8	10	6	-
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	16	32	16	32	-
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	-
Jan. 1930.	Haemolytic Reaction.	5.0	10.0	5.0	5.0	10.0
	Bactericidal reaction with *B. suipestifer*	6	4	4	5	4
	Bactericidal reaction with *B. coli* "X"	2	0	2	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	64	32	32	32
	Agglutination reaction with *B. abortus* (Hog)	32	256	64	64	128
Mar.	Haemolytic Reaction.	5.0	10.0	6.6	10.0	5.0
	Bactericidal reaction with *B. suipestifer*	5	4	4	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	64	8	32	16
	Agglutination reaction with *B. abortus* (Hog)	64	64	128	128	256
May.	Haemolytic Reaction.	5.0	5.0	5.0	5.0	5.0
	Bactericidal reaction with *B. suipestifer*	4	-	4	-	2
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	32	16	32	16
	Agglutination reaction with *B. abortus* (Hog)	64	128	-	32	64
Sept.	Haemolytic Reaction.	2.8	-	* 4.0	-	4.0
	Bactericidal reaction with *B. suipestifer*	4	-	5	-	4
	Bactericidal reaction with *B. coli* "X"	2	-	2	-	0
	Bactericidal reaction with *B. coli* "F_3"	0	-	0	-	0
	Agglutination reaction with *B. paratyphosus B.*	64	-	32	-	64
	Agglutination reaction with *B. abortus* (Hog)	16	-	64	-	16

ASHTOWN EXPERIMENT 1929-30.

GROUP VIII - OUTDOOR.

	Number.	131.	181.	148.	171.	136.
Dec. 1929	Haemolytic Reaction.	-	-	4.0	5.0	5.0
	Bactericidal reaction with *B. suipestifer*	-	8	9	9	8
	Bactericidal reaction with *B. coli* "X"	-	-	-	-	-
	Agglutination reaction with *B. paratyphosus B.*	-	32	8	16	8
	Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	-
Jan. 1930.	Haemolytic Reaction.	5.0	5.0 ,	5.0	10.0	13.3
	Bactericidal reaction with *B. suipestifer*	6	5	4	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	4	0	0
	Agglutination reaction with *B. paratyphosus B.*	16	64	64	128	256
	Agglutination reaction with *B. abortus* (Hog)	32	64	64	64	128
Mar.	Haemolytic Reaction.	6.6	5.0	10.0	10.0	10.0
	Bactericidal reaction with *B. suipestifer*	5	4	5	4	4
	Bactericidal reaction with *B. coli* "X"	2	0	0	0	2
	Agglutination reaction with *B. paratyphosus B.*	64	64	16	32	16
	Agglutination reaction with *B. abortus* (Hog)	128	128	128	256	128
May.	Haemolytic Reaction.	5.0	3.3	4.0	4.0	5.0
	Bactericidal reaction with *B. suipestifer*	4	-	-	4	4
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	2
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	8	32	8	16	-
	Agglutination reaction with *B. abortus* (Hog)	64	32	64	-	64
Sept.	Haemolytic Reaction.	4.0	3.3	2.8	4.0	5.0 *
	Bactericidal reaction with *B. suipestifer*	4	5	4	4	5
	Bactericidal reaction with *B. coli* "X"	0	0	0	0	2
	Bactericidal reaction with *B. coli* "F_3"	0	0	0	0	0
	Agglutination reaction with *B. paratyphosus B.*	32	64	64	64	16
	Agglutination reaction with *B. abortus* (Hog)	8	32	64	32	64

PROTOCOLS

OF

GARROCHORAN EXPERIMENT.

Results of Haemolytic Reaction and Titration of Complement are given in M.H.D.s per c.c.

" " Bactericidal Reactions are given in Bactericidal Units.

" " Agglutination Reactions are given in Agglutination Units.

DATES OF SAMPLING AND ANIMALS SAMPLED.

1930.	May	7.	20 wethers and barren ewes.	5 from each heft.
		27.	20 ewes.	5 from each heft.
	July	7.	20 wethers and barren ewes,	5 from each heft.
		15.	20 ewes.	5 from each heft.
	Oct.	13.	20 ewes.	5 from each heft.
		15.	20 ewes.	5 from each heft.
		22.	20 ewes.	5 from each heft.
		27.	19 gimmers.	(5 from hefts II,III & IV. (4 from heft I.
		29.	20 hoggs.	5 from each heft.
	Nov.	3.	20 barren ewes.	5 from each heft.
		6.	20 hoggs.	5 from each heft.
1931.	Jan.	19.	20 ewes.	5 from each heft.
		21.	20 ewes.	5 from each heft.
		26.	20 ewes.	5 from each heft.
		28.	20 ewes.	5 from each heft.
	Feb.	2.	19 gimmers	(5 from hefts II,III & IV. (4 from heft I.
		4.	19 hoggs 1 gimmer (heft IV)	(5 from hefts I,II & III. (4 from heft IV.
		9.	20 barren ewes.	5 from each heft.
		11.	20 hoggs.	5 from each heft.
	Mar.	23.	20 ewes.	5 from each heft.
		25.	20 ewes	5 from each heft.
		30.	20 ewes.	10 from each of hefts II & III
	Apl.	1.	20 ewes.	10 from each of hefts I & II.
		6.	(10 gimmers.	4 from heft I, 6 from heft IV.
			(10 hoggs.	5 from each of hefts I & IV.
		8.	(10 gimmers.	
			(10 hoggs.	5 from each of hefts II & III.
		13.	(10 barren ewes.	
			(10 hoggs.	5 from each of hefts II & IV.
		15.	(10 barren ewes.	
			(10 hoggs.	5 from each of hefts I & III.
	May	21.	20 ewes.	5 from each heft.
		23.	20 ewes.	5 from each heft.
		27.	20 ewes.	5 from each heft.
		29.	20 ewes.	5 from each heft.
	June	2.	20 gimmers.	5 from each heft.
		4.	20 hoggs.	5 from each heft.
		8.	19 hoggs.	(5 from heft I, II & III. (4 from heft IV.
	July	20.	20 ewes.	5 from each heft.
		22.	20 ewes.	5 from each heft.
	Nov.	2.	20 ewes	5 from each heft.
		5.	20 ewes.	5 from each heft.

9 /

1931.	Nov.	9.	20 ewes.	5 from each heft.
		11.	20 ewes.	5 from each heft.
		17.	20 ewes.	5 from each heft.
		18.	20 ewes.	5 from each heft.
		23.	20 ewes.	5 from each heft.
		25.	20 ewes.	5 from each heft.
1932.	Mar.	21.	20 ewes.	5 from each heft.
		23.	20 ewes.	5 from each heft.
		28.	20 ewes.	5 from each heft.
		30.	20 ewes.	5 from each heft.
	Apl.	11.	20 ewes.	5 from each heft.
		13.	20 ewes.	5 from each heft.

GARROCHORAN EXPERIMENT.

EWES. Heft I.

	13/10/30.					15/10/30.					22/10/30.				
Number.	3.	13.	38.	52.	79.	2.	26.	29.	31.	61.	18.	23.	33.	49.	62.
Haemolytic Reaction.	3.3	2.8	3.3	2.5	1.8	2.8	2.2	3.3	1.8	2.5	1.6	2.0	1.6	2.0	1.6
Bactericidal reaction with B. suipestifer	2	2	2	2	0	4	4	4	4	4	2	2	2	2	2
Bactericidal reaction with B. coli "X"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bactericidal reaction with B. coli "F_3"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	128	32	64	8	32	64	64	128	128	16	64	32	128	64	128
Agglutination reaction with B. abortus (Hog)	128	>256	>256	64	16	256	256	128	64	64	-	-	-	-	-

	19/1/31.					21/1/31.					26/1/31.					28/1/31.				
Number.	3.	13.	38.	52.	79.	2.	26.	29.	31.	61.	9.	40.	47.	69.	83.	18.	23.	33.	49.	62.
Haemolytic Reaction.	2.0	1.5	1.5	1.2	1.4	2.0	2.0	2.2	2.2	2.2	3.3	2.0	2.2	2.0	1.8	2.2	2.2	1.3	1.2	1.6
Bactericidal reaction with B. suipestifer	4	4	4	6	4	2	2	4	4	2	2	2	4	2	2	6	6	6	6	6
Bactericidal reaction with B. coli "X"	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0	0	2	0	2	0	-	-	-	-	-	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	64	64	128	32	128	32	16	64	16	8	16	16	16	32	32	16	16	64	32	16
Agglutination reaction with B. abortus (Hog)	64	256	256	64	128	256	256	128	32	128	64	64	64	64	64	256	256	256	256	256

	23/3/31.					25/3/31.					1/4/31.					29/5/31.				
Number.	3.	13.	38.	52.	79.	2.	26.	29.	31.	61.	9.	18.	23.	33.	40.	47.	49.	62.	69.	83.
Haemolytic Reaction.	1.5	1.5	1.8	1.8	2.2	1.8	2.2	2.2	2.2	2.2	1.8	1.5	1.5	1.2	2.0	1.8	1.8	1.8	1.5	1.6
Bactericidal reaction with B. suipestifer	4	4	4	4	4	2	2	2	2	2	4	4	4	4	2	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	-	-	-	-	-	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	32	32	16	32	32	16	128	64	32	8	8	32	64	8	32	16	8	32	64
Agglutination reaction with B. abortus (Hog)	32	128	256	64	-	>256	128	8	128	128	16	64	64	128	32	32	32	32	32	32

	21/5/31.					23/5/31.					27/5/31.					29/5/31.				
Number.	9.	13.	25.	69.	79.	48	53.	55.	56.	68.	23.	29.	47.	58.	70.	4.	14.	31.	67.	
Haemolytic Reaction.	4.0	2.8	5.0	3.3	2.0	2.8	5.0	4.0	4.0	2.2	2.2	2.2	2.2	2.5	2.8	3.3	3.3	3.3	3.3	2.8
Bactericidal reaction with B. suipestifer	2	2	2	2	2	2	2	2	2	2	4	4	4	4	4	5	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0	2	2	2	0	0	0	0	2	0	0	0	0	0	2	2
Bactericidal reaction with Str. haemolyticus	-	-	-	-	-	0	0	2	2	0	-	-	-	-	-	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	16	32	16	16	32	32	64	32	16	16	16	32	32	8	16	64	32	32	64
Agglutination reaction with B. abortus (Hog)	32	64	64	64	128	64	64	64	128	128	64	128	128	256	32	128	256	128	128	64

	20/7/31.					22/7/31.				
Number.	13.	38.	47.	79.	82.	14.	22.	25.	31.	40.
Haemolytic Reaction.	3.3	2.5	2.0	2.2	2.0	3.3	4.0	5.0	4.0	3.3
Titration of Complement.	-	-	-	-	-	16.6	16.6	20.0	16.6	25.0
Bactericidal reaction with B. suipestifer	4	2	4	4	4	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	2	2	0	0	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	2	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	32	32	32	64	16	4	8	32	16
Agglutination reaction with B. abortus (Hog)	16	64	32	32	16	8	16	128	256	256

GARROCHORAN

EWES.

EXPERIMENT.

HEFT I (Ctd.)

2/11/31.

Number.	12.	31.	48.	63.	85.
Haemolytic Reaction.	2.8	2.8	2.2	2.5	2.8
Titration of Complement.	6.6	10.0	5.0	5.0	6.6
Bactericidal reaction with *B. coli* "X"	0	0	0	1	0
Bactericidal reaction with *Str. haemolyticus*	-	-	-	-	-
Agglutination reaction with *B. abortus* (Hog)	64	64	128	256	128

17/11/31.

Number.	11.	56.	69.	77.	82.
Haemolytic Reaction.	2.0	3.3	2.5	2.2	2.5
Titration of Complement.	5.0	6.6	6.6	5.0	6.6
Bactericidal reaction with *B. coli* "X"	1	1	-	-	-
Bactericidal reaction with *Str. haemolyticus*	0	0	2	2	0
Agglutination reaction with *B. abortus* (Hog)	64	64	64	64	32

21/3/32.

Number.	12.	31.	48.	63.	65.
Haemolytic Reaction.	2.8	5.0	4.0	3.3	2.8
Titration of Complement.	10.0	5.0	5.0	5.0	13.3
Bactericidal reaction with *B. coli* "X"	3	2	4	2	2
Bactericidal reaction with *Str. haemolyticus*	5	3	3	3	2
Agglutination reaction with *B. abortus* (Hog)	256	128	128	256	256

Number.	11.	26.	56.	78.	82.
Haemolytic Reaction.	2.8	3.3	2.5	3.3	2.8
Titration of Complement.	6.6	6.6	6.6	10.0	6.6
Bactericidal reaction with *B. coli* "X"	3	2	2	5	2
Bactericidal reaction with *Str. haemolyticus*	1	1	1	1	1
Agglutination reaction with *B. abortus* (Hog)	64	256	128	64	64

5/11/31.

23.	53.	58.	60.	70.
2.8	2.2	2.2	2.2	3.3
5.0	6.6	6.6	6.6	6.6
0	0	0	0	2
1	3	2	1	5
128	64	128	128	128

18/11/31.

26.	47.	55.	78.	89.
1.8	2.2	2.2	1.8	2.2
5.0	5.0	5.0	5.0	13.3
0	1	1	1	0
3	4	3	3	-
256	128	128	128	128

23/3/32.

23.	53.	58.	60.	70.
2.8	2.2	2.5	2.8	2.8
5.0	5.0	4.0	5.0	5.0
2	4	1	2	1
0	0	0	0	2
64	32	64	256	32

13/4/32.

475.	480.	490.	491.	495.
2.5	2.8	3.3	2.8	2.2
4.0	3.3	5.0	4.0	5.0
0	1	2	2	0
-	3	0	0	0
128	128	128	256	128

9/11/31.

16.	62.	73.	83.	85.
1.8	1.5	1.3	1.6	2.2
6.6	4.0	4.0	4.0	4.0
0	0	0	0	0
-	0	2	3	3
16	128	128	64	128

23/11/31.

38.	68.	486.	495.
1.8	1.8	2.2	2.2
13.3	20.0	20.0	20.0
1	1	1	1
4	3	3	2
256	128	128	128

28/3/32.

16.	62.	73.	83.	85.
2.2	2.8	2.2	2.8	2.2
10.0	10.0	10.0	10.0	13.3
2	0	1	1	1
-	3	2	2	2
128	128	256	128	128

11/11/31.

2.	22.	66.	81.	88.
-	-	-	-	-
-	-	-	-	-
0	1	1	1	0
2	3	-	3	-
256	256	128	128	256

25/11/31.

475.	480.	484.	488.	490.	491.
2.8	2.8	2.8	4.0	3.3	2.5
5.0	5.0	5.0	6.6	5.0	5.0
1	1	1	0	2	1
2	0	0	0	0	0
128	64	32	128	64	128

30/3/32.

2.	22.	66.	81.	88.
3.3	2.8	2.8	3.3	3.3
6.6	10.0	13.3	10.0	13.3
2	0	2	1	0
-	3	-	0	0
>256	128	128	128	128

GARROCHORAN.

EWES.

	13/10/30.				
Number.	104.		223.	232.	244.
Haemolytic Reaction.	3.3		3.3	2.0	1.5
Bactericidal reaction with B. suipestifer	2		2	2	3
Bactericidal reaction with B. coli "X"	0		0	0	0
Bactericidal reaction with B. coli "F_3"	0		0	0	0
Agglutination reaction with B. paratyphosus B.	64		16	8	16
Agglutination reaction with B. abortus (Hog)	>256		64	64	128

	19/1/31.				
Number.	104.	147.	223.	232.	244.
Haemolytic Reaction.	1.3	1.3	1.5	1.2	1.3
Bactericidal reaction with B. suipestifer	2	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	32	32	16	64	32
Agglutination reaction with B. abortus (Hog)	256	64	64	128	64

	23/3/31.				
Number.	104.	147.	223.	232.	244.
Haemolytic Reaction.	1.8	1.8	1.8	1.3	1.8
Bactericidal reaction with B. suipestifer	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	-	-	-	-	-
Agglutination reaction with B. paratyphosus B.	64	64	32	32	128
Agglutination reaction with B. abortus (Hog)	-	32	64	64	64

	21/5/31.				
Number.	94.	144.	148.	215.	232.
Haemolytic Reaction.	4.0	3.3	2.5	2.8	2.5
Bactericidal reaction with B. suipestifer	2	2	2	2	2
Bactericidal reaction with B. coli "X"	0	2	0	0	0
Bactericidal reaction with Str. haemolyticus	-	-	-	-	-
Agglutination reaction with B. paratyphosus B.	16	32	32	64	16
Agglutination reaction with B. abortus (Hog)	64	32	64	64	32

	20/7/31.				
Number.	144.	148.	217.	223.	232.
Haemolytic Reaction.	2.5	2.5	1.6	2.2	2.5
Titration of Complement.	13.3	13.3	11.1	25.0	25.0
Bactericidal reaction with B. suipestifer.	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	2	0	0	0
Bactericidal reaction with Str. haemolyticus	2	2	0	0	0
Agglutination reaction with B. paratyphosus B.	16	32	32	16	8
Agglutination reaction with B. abortus (Hog)	64	32	128	16	64

EXPERIMENT.

Heft II.

	15/10/30.				
	94.	108.	120.	137.	161.
Haemolytic Reaction.	2.0	2.8	2.2	2.2	2.2
Bactericidal reaction with B. suipestifer	5	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	64	64	32	64	16
Agglutination reaction with B. abortus (Hog)	128	256	128	64	128

	21/1/31.					26/1/31.				
	94.	108.	120.	137.	161.	148.	188.	216.	217.	220.
Haemolytic Reaction.	2.2	1.5	1.5	2.2	2.2	1.8	2.5	2.0	2.2	2.2
Bactericidal reaction with B. suipestifer	4	4	4	4	4	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	-	-	0	-	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0	-	-	-	0	0
Agglutination reaction with B. paratyphosus B.	16	16	4	32	32	16	8	32	32	4
Agglutination reaction with B. abortus (Hog)	256	64	64	64	64	128	64	64	128	32

	25/3/31.					30/3/31.				
	94.	108.	120.	137.	161.	141.	144.	148.	149.	188.
Haemolytic Reaction.	2.5	1.8	2.2	1.8	1.8	1.5	1.6	1.3	1.3	2.0
Bactericidal reaction with B. suipestifer	2	3	2	2	2	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0	0	0	2	0	0
Agglutination reaction with B. paratyphosus B.	64	64	32	16	32	16	32	32	32	16
Agglutination reaction with B. abortus (Hog)	256	64	64	16	64	64	64	32	128	32

	23/5/31.					27/5/31.				
	101.	124.	149.	153.	223.	182.	204.	211.	219.	242.
Haemolytic Reaction.	2.8	4.0	2.8	3.3	3.3	2.5	2.5	1.8	2.0	2.8
Bactericidal reaction with B. suipestifer	2	2	2	2	2	4	4	4	4	5
Bactericidal reaction with B. coli "X"	2	2	2	2	0	2	2	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0	-	-	-	-	-
Agglutination reaction with B. paratyphosus B.	64	16	64	16	16	16	16	16	8	8
Agglutination reaction with B. abortus (Hog)	128	64	64	16	64	128	128	128	128	32

	22/7/31.				
	94.	101.	110.	120.	149.
Haemolytic Reaction.	3.3	2.8	2.8	2.5	2.2
Titration of Complement.	16.6	16.6	20.0	40.0	25.0
Bactericidal reaction with B. suipestifer.	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	8	32	16	32
Agglutination reaction with B. abortus (Hog)	128	128	128	128	128

	22/10/30.				
	144.	149.	165.	234.	246.
Haemolytic Reaction.	2.5	1.6	2.2	1.8	2.2
Bactericidal reaction with B. suipestifer	2	2	2	3	2
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	64	16	32	32
Agglutination reaction with B. abortus (Hog)	-	-	-	-	-

	28/1/31.				
	141.	144.	149.	234.	246.
Haemolytic Reaction.	1.8	2.2	1.3	2.5	1.5
Bactericidal reaction with B. suipestifer	6	6	6	6	6
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	32	8	32	64	32
Agglutination reaction with B. abortus (Hog)	128	128	32	256	128

	216.	217.	220.	234.	246.
Haemolytic Reaction.	1.5	1.5	1.8	2.2	2.2
Bactericidal reaction with B. suipestifer	4	4	4	4	2
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	2	0	0
Agglutination reaction with B. paratyphosus B.	16	32	128	16	32
Agglutination reaction with B. abortus (Hog)	128	128	64	128	64

	29/5/31.				
	120.	178.	191.	206.	209.
Haemolytic Reaction.	2.8	2.8	2.8	2.8	2.2
Bactericidal reaction with B. suipestifer	2	4	5	5	5
Bactericidal reaction with B. coli "X"	0	0	2	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	64	64	16	32	16
Agglutination reaction with B. abortus (Hog)	64	64	64	32	32

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EWES.

EXPERIMENT.

Heft II (Ctd.)

		2/11/31.				5/11/31.					9/11/31.					11/11/31.				
Number.		177.	182.	205.	206.	244.	124.	178.	191.	220.	224.	133.	195.	204.	223.	234.	107.	121.	187.	217.	240.
Haemolytic Reaction.		2.8	1.8	1.8	2.2	2.5	1.8	3.3	2.2	2.8	2.8	1.8	1.5	1.8	2.0	2.5	-	-	-	-	-
Titration of Complement.		6.6	10.0	5.0	10.0	10.0	6.6	6.6	10.0	10.0	10.0	2.8	4.0	4.0	4.0	6.6	-	-	-	-	-
Bactericidal reaction with	B. coli "X"	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	1	0	1	1	1
	Str. haemolyticus	-	-	-	-	-	2	2	1	-	-	2	-	2	-	0	1	1	2	2	2
Agglutination reaction with	B. abortus (Hog)	64	128	64	32	64	32	32	64	32	32	64	64	256	32	32	128	256	128	128	256

		17/11/31.				18/11/31.					23/11/31.					25/11/31.				
Number.		161.	171.	215.	216.	242.	246.	501.	508.	509.	529.	95.	111.	149.	197.	211.	183.	502.	524.	528.	539.
Haemolytic Reaction		2.8	2.5	3.3	2.2	2.0	2.2	2.0	2.5	3.3	2.5	1.5	2.2	1.5	3.3	1.5	2.2	4.0	3.3	5.0	3.3
Titration of Complement.		4.0	5.0	4.0	4.0	4.0	6.6	5.0	5.0	10.0	6.6	10.0	10.0	6.6	10.0	10.0	6.6	10.0	6.6	10.0	5.0
Bactericidal reaction with	B. coli "X"	0	2	4	0	2	0	1	1	0	0	0	1	1	1	2	1	2	2	2	3
	Str. haemolyticus	-	0	1	1	1	3	2	2	2	2	-	-	-	-	2	0	0	0	0	2
Agglutination reaction with	B. abortus (Hog)	64	128	128	64	64	64	128	128	128	128	64	128	128	64	64	64	128	128	64	16

		21/3/32.				23/3/32.					28/3/32.					30/3/32.				
Number.		177.	182.	205.	206.	244.	124.	178.	191.	220.	224.	133.	195.	204.	223.	234.	107.	121.	187.	217.	240.
Haemolytic Reaction.		3.3	2.2	2.2	2.5	2.2	2.0	2.2	2.0	2.0	2.0	2.0	2.5	2.5	2.2	2.5	3.3	3.3	2.8	2.8	3.3
Titration of Complement.		10.0	10.0	6.6	10.0	13.3	4.0	5.0	5.0	5.0	5.0	10.0	10.0	10.0	10.0	6.6	6.6	6.6	6.6	6.6	6.6
Bactericidal reaction with	B. coli "X"	1	1	3	4	2	1	1	0	1	2	0	0	1	1	2	1	2	0	2	1
	Str. haemolyticus	4	2	4	2	2	0	0	2	0	2	2	2	2	-	2	2	-	-	2	2
Agglutination reaction with	B. abortus (Hog)	64	128	64	32	128	64	16	16	64	8	128	128	128	128	128	128	128	128	256	128

		11/4/32.				13/4/32.				
Number.		111.	161.	171.	183.	197.	501.	502.	509.	515.	539.
Haemolytic Reaction.		3.3	3.3	3.3	1.8	5.0	2.5	2.5	3.3	2.2	2.5
Titration of Complement.		5.0	6.6	10.0	6.6	6.6	4.0	4.0	5.0	4.0	4.0
Bactericidal reaction with	B. coli "X"	2	2	0	0	0	0	0	0	1	1
	Str. haemolyticus	1	1	1	2	1	0	2	2	-	0
Agglutination reaction with	B. abortus (Hog)	32	32	64	64	64	128	128	32	64	128

GARROCHORAN

EWES.

	13/10/30.				
Number.	271.	292.	329.	344.	357.
Haemolytic Reaction.	1.5	2.8	3.3	2.5	2.2
Bactericidal reaction with B. suipestifer	2	2	2	2	0
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with B. coli "F_3"	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	64	16	8	16	64
Agglutination reaction with B. abortus (Hog)	64	64	>256	32	16

	19/1/31.				
Number.	271.	292.	329.	344.	357.
Haemolytic Reaction.	1.2	1.2	1.3	1.3	1.2
Bactericidal reaction with B. suipestifer	4	2	2	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	-	-	-
Agglutination reaction with B. paratyphosus B.	16	128	128	64	128
Agglutination reaction with B. abortus (Hog)	256	64	64	128	64

	23/3/31.				
Number.	271.	292.	329.	344.	357.
Haemolytic Reaction.	1.3	2.2	2.5	2.2	2.2
Bactericidal reaction with B. suipestifer	5	5	4	5	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	-	0	-	-	-
Agglutination reaction with B. paratyphosus B.	32	32	32	64	32
Agglutination reaction with B. abortus (Hog)	128	64	64	64	64

	21/5/31.				
Number.	262.	271.	312.	331.	355.
Haemolytic Reaction.	3.3	1.8	1.8	2.2	2.8
Bactericidal reaction with B. suipestifer	2	0	2	2	2
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str.haemolyticus	-	-	-	-	-
Agglutination reaction with B. paratyphosus B.	16	32	32	32	32
Agglutination reaction with B. abortus (Hog)	128	128	-	-	-

	20/7/31.				
Number.	271.	337.	339.	355.	371.
Haemolytic Reaction.	1.8	2.8	1.6	3.3	2.2
Titration of Complement.	11.1	16.6	20.0	16.6	40.0
Bactericidal reaction with B. suipestifer	4	4	4	5	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	2	0	2	0
Agglutination reaction with B. paratyphosus B.	16	8	32	32	16
Agglutination reaction with B. abortus (Hog)	16	256	128	32	16

EXPERIMENT.

Heft III.

	15/10/30.				
	267.	283.	334.	338.	363.
	2.2	2.5	2.2	2.2	2.2
	4	5	4	4	4
	0	0	0	0	0
	0	0	0	0	0
	64	64	32	16	16
	128	128	128	128	128

	21/1/31.				
	267.	283.	334.	338.	363.
	2.5	2.2	2.2	1.8	1.8
	2	4	2	4	4
	0	0	-	0	0
	0	0	0	0	0
	16	16	16	8	16
	128	64	128	32	128

	25/3/31.				
	267.	285.	334.	338.	363.
	1.8	1.8	2.5	2.8	1.8
	2	3	2	2	2
	0	0	0	0	0
	0	0	0	2	2
	16	32	32	16	16
	128	64	128	32	64

	23/5/31.				
	284.	287.	344.	363.	376.
	2.8	2.8	2.0	2.8	4.0
	2	2	2	2	2
	2	2	2	0	2
	-	0	2	0	2
	16	64	64	32	64
	64	64	64	32	64

	22/7/31.				
	262.	284.	331.	344.	357.
	4.0	2.2	2.2	2.0	2.5
	16.6	40.0	25.0	16.6	20.0
	4	4	2	4	4
	2	0	0	0	0
	0	0	2	0	0
	16	32	32	16	64
	64	16	64	128	256

	26/1/31.				
	287.	293.	320.	331.	355.
	2.5	2.0	2.2	2.2	1.8
	4	4	4	2	2
	0	0	0	0	0
	0	0	0	0	0
	16	16	8	16	32
	64	64	16	64	256

	30/3/31.				
	262.	284.	287.	293.	312.
	2.8	1.8	2.8	2.2	2.5
	4	4	4	4	4
	0	0	0	0	0
	0	4	0	2	0
	-	128	16	8	8
	32	32	16	128	128

	27/5/31.				
	269.	335.	338.	357.	371.
	2.5	2.2	2.0	1.8	2.2
	4	4	4	4	4
	0	0	2	2	2
	-	-	-	-	-
	32	16	16	16	128
	128	64	16	128	64

	22/10/30.				
	262.	284.	312.	335.	343.
	1.8	2.0	2.2	2.2	1.8
	2	0	2	2	2
	0	0	0	0	0
	0	0	0	0	0
	16	128	16	64	128
	-	-	-	-	-

	28/1/31.				
	262.	284.	312.	335.	343.
	2.0	1.3	1.5	2.0	2.0
	6	6	6	6	4
	0	0	0	2	0
	0	0	-	0	0
	32	32	32	-	64
	128	64	128	-	64

	30/3/31.				
	320.	331.	335.	343.	355.
	2.5	1.8	1.5	1.8	2.2
	4	4	4	4	5
	0	0	0	0	0
	2	2	0	0	-
	4	16	16	16	16
	128	64	64	128	256

	29/5/31.				
	263.	285.	320.	334.	384.
	2.8	3.3	3.3	2.8	2.8
	5	5	5	5	5
	2	0	2	0	2
	0	0	0	0	0
	64	64	16	32	16
	64	64	64	128	128

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EWES.

EXPERIMENT.

Heft III. (Ctd.)

	2/11/31.					5/11/31.					9/11/31.					11/11/31.				
Number.	331.	343.	344.	354.	359.	267.	337.	357.	371.	384.	293.	334.	355.	363.	375.	336.	339.	341.	348.	376.
Haemolytic Reaction.	2.2	2.5	2.2	2.2	2.8	2.2	3.3	2.2	2.5	1.8	2.2	2.5	1.8	1.5	2.2	-	-	-	-	-
Titration of Complement.	10.0	6.6	10.0	6.6	5.0	6.6	5.0	6.6	10.0	6.6	4.0	4.0	6.6	2.8	6.6	-	-	-	-	-
Bactericidal (B. coli "X"	0	1	1	0	0	0	0	1	1	0	0	2	0	0	0	0	1	0	1	1
reaction with(Str. haemolyticus	-	-	-	-	-	-	-	3	1	-	2	3	-	2	-	3	2	2	2	2
Agglutination(B. abortus (Hog)	64	64	64	32	64	32	128	64	64	32	128	256	256	128	32	256	256	128	128	128
reaction with(																				

	17/11/31.					18/11/31.					23/11/31.					25/11/31.				
Number.	263.	282.	291.	329.	378.	547.	550.	556.	560.	573.	260.	335.	364.	376.	382.	356.	546.	555.	561.	565.
Haemolytic Reaction.	2.2	2.2	2.5	1.8	2.0	1.8	2.8	2.5	2.8	1.8	2.2	2.2	2.8	2.2	1.8	4.0	2.8	4.0	3.3	3.3
Titration of Complement.	4.0	5.0	6.6	10.0	6.6	10.0	10.0	6.6	10.0	5.0	13.3	10.0	20.0	13.3	10.0	6.6	6.6	20.0	5.0	5.0
Bactericidal (B. coli "X"	2	1	0	0	0	3	0	0	1	0	2	1	0	0	1	1	1	3	1	1
reaction with(Str. haemolyticus	-	-	1	1	1	-	-	3	2	1	4	4	3	-	-	2	2	2	2	0
Agglutination(B. abortus (Hog)	64	64	64	128	64	64	128	64	64	64	128	64	128	128	64	64	64	256	64	64
reaction with(																				

	21/3/32.					23/3/32.					28/3/32.					30/3/32.				
Number.	331.	343.	344.	354.	359.	267.	337.	357.	371.	384.	293.	334.	355.	369.	375.	336.	339.	341.	348.	370.
Haemolytic Reaction.	2.2	2.2	4.0	2.5	3.3	2.0	2.5	1.8	2.2	1.5	2.5	2.8	3.3	2.2	2.5	3.3	2.5	3.3	3.3	2.5
Titration of Complement.	6.6	10.0	20.0	13.3	10.0	4.0	5.0	5.0	5.0	4.0	10.0	13.3	10.0	10.0	10.0	10.0	6.6	6.6	6.6	6.6
Bactericidal (B. coli "X"	2	2	0	3	1	3	0	2	0	0	1	4	1	2	1	2	2	2	2	3
reaction with(Str. haemolyticus	2	2	3	2	0	2	2	2	2	0	2	2	3	5	2	-	2	0	0	-
Agglutination(B. abortus (Hog)	64	128	256	256	128	128	32	128	64	32	128	128	256	128	32	128	64	64	64	64
reaction with(																				

	11/4/32.					13/4/32.				
Number.	263.	291.	329.	335.	364.	550.	555.	560.	565.	473.
Haemolytic Reaction.	2.8	3.3	4.0	2.2	2.8	3.3	2.8	4.0	2.2	1.8
Titration of Complement.	5.0	5.0	10.0	5.0	6.6	5.0	5.0	4.0	4.0	3.3
Bactericidal (B. coli "X"	3	5	5	3	0	1	1	1	0	1
reaction with(Str. haemolyticus	1	1	1	2	1	-	-	0	-	-
Agglutination(B. abortus (Hog)	256	64	64	32	128	>256	128	64	64	128
reaction with(										

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EWES.

EXPERIMENT.

Heft IV.

13/10/30.

Number.	413.	420.	437.	441.	456.
Haemolytic Reaction.	2.2	2.5	2.2	2.2	2.2
Bactericidal reaction with B. suipestifer	2	2	2	2	0
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with B. coli "F_3"	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	16	32	32	64
Agglutination reaction with B. abortus (Hog)	32	128	128	128	128

19/1/31.

Number.	411.	420.	437.	441.	456.
Haemolytic Reaction.	1.2	1.5	1.3	1.3	1.3
Bactericidal reaction with B. suipestifer	4	2	2	2	2
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	2	0	0	0
Agglutination reaction with B. paratyphosus B.	64	64	64	64	64
Agglutination reaction with B. abortus (Hog)	64	128	128	128	128

23/3/31.

Number.	411.	420.	437.	456.	459.
Haemolytic Reaction.	1.8	1.3	1.6	1.8	1.3
Bactericidal reaction with B. suipestifer	4	4	5	5	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	-	-	-	-	-
Agglutination reaction with B. paratyphosus B.	32	32	32	128	16
Agglutination reaction with B. abortus (Hog)	64	64	128	64	64

21/5/31.

Number.	397.	413.	420.	438.	444.
Haemolytic Reaction.	2.2	2.5	2.2	3.3	3.3
Bactericidal reaction with B. suipestifer	2	2	2	2	0
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	-	-	-	-	-
Agglutination reaction with B. paratyphosus B.	16	16	64	16	32
Agglutination reaction with B. abortus (Hog)	-	32	32	64	32

20/7/31.

Number.	395.	414.	418.	444.	455.
Haemolytic Reaction.	3.3	2.2	3.3	2.5	3.3
Titration of Complement.	11.1	16.6	20.0	16.6	40.0
Bactericidal reaction with B. suipestifer	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	2	2
Agglutination reaction with B. paratyphosus B.	16	8	32	32	16
Agglutination reaction with B. abortus (Hog)	16	256	128	32	16

15/10/30.

419.	421.	444.	454.	459.
2.2	2.2	2.5	2.2	2.2
4	4	4	4	4
0	0	0	0	0
0	0	0	0	0
16	32	32	32	64
256	128	128	32	64

21/1/31.

419.	421.	444.	454.	459.
1.8	1.8	2.5	1.8	1.5
4	2	2	4	2
0	0	0	2	0
-	0	0	0	0
16	8	16	8	8
128	64	128	64	64

25/3/31.

419.	421.	444.	454.	441. (sick)
1.5	1.8	1.8	1.3	1.3
2	2	2	2	2
0	0	0	0	0
0	0	0	0	0
32	8	32	32	32
128	32	128	16	64

23/5/31.

408.	414.	428.	437.	442.
3.3	2.5	4.0	2.2	2.8
2	3	2	2	2
2	0	2	2	0
0	0	0	0	0
64	32	32	64	64
128	256	64	64	64

22/7/31.

397.	408.	420.	433.	437.
2.2	2.8	2.2	3.3	2.2
13.3	20.0	20.0	20.0	20.0
4	4	4	4	4
0	0	0	0	0
0	0	2	2	0
16	32	16	32	32
64	256	128	-	128

26/1/31.

397.	408.	422.	447.	450.
1.5	2.2	2.2	1.3	1.8
4	4	2	4	4
0	0	0	0	0
0	0	0	0	0
16	16	16	16	8
128	256	128	128	64

1/4/31.

397.	400.	406.	408.	413.
1.2	1.3	1.2	1.3	1.3
4	4	4	4	4
2	0	0	0	0
0	0	0	0	0
16	64	64	16	16
16	8	32	64	16

27/5/31.

422.	425.	432.	433.	436.
2.0	3.3	3.3	2.8	4.0
4	4	4	5	4
0	2	2	0	2
-	-	-	-	-
16	32	16	16	64
32	64	64	128	32

22/10/30.

400.	406.	413.	428.	438.
2.2	2.0	1.8	2.0	2.5
2	2	2	2	2
0	0	0	0	0
0	0	0	0	0
128	128	64	64	128
-	-	-	-	-

28/1/31.

400.	406.	413.	428.	438.
1.5	1.4	1.3	1.5	1.5
6	6	6	6	6
0	0	0	0	0
0	2	0	2	2
64	64	16	16	32
64	128	>256	256	>256

29/5/31.

409.	423.	435.	443.	446.
2.2	3.3	3.3	2.8	2.2
5	5	4	5	5
0	0	0	0	0
0	0	0	0	0
64	32	32	16	16
128	32	64	64	64

422. 428. 438. 447. 450.
1.3 1.6 1.6 1.0 1.5
4 4 2 4 4
0 2 0 0 2
0 - - 0 0
32 16 32 16 8
32 32 16 16 32

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EWES.

EXPERIMENT.

Heft IV. (Ctd.)

	2/11/31.					5/11/31.					9/11/31.					11/11/31.				
Number.	408.	422.	426.	435.	445.	411.	420.	437.	438.	440.	414.	424.	442.	447.	458.	401.	403.	418.	443.	446.
Haemolytic Reaction.	2.2	1.8	1.5	2.0	2.2	1.5	2.2	1.8	2.5	1.8	2.2	2.5	1.5	1.5	1.5	-	-	-	-	-
Titration of Complement.	5.0	6.6	5.0	5.0	5.0	6.6	6.6	6.6	4.0	5.0	4.0	4.0	4.0	2.8	4.0	-	-	-	-	-
Bactericidal reaction with B. coli "X"	0	0	0	1	1	0	2	2	1	2	0	0	0	0	0	1	1	1	1	1
Bactericidal reaction with Str. haemolyticus	-	-	-	-	-	-	3	1	-	-	-	2	-	-	-	0	-	-	2	2
Agglutination reaction with B. abortus (Hog)	32	64	64	64	128	16	64	32	64	64	128	32	128	64	128	128	256	256	256	256

	17/11/31.					18/11/31.					23/11/31.					25/11/31.				
Number.	433.	450.	451.	453.	459.	590.	583.	586.	589.	592.	399.	409.	434.	444.	456.	432.	454.	577.	580.	584.
Haemolytic Reaction.	1.8	2.0	2.2	2.0	1.5	2.8	2.5	1.5	2.5	2.2	2.2	2.3	2.2	2.2	2.5	4.0	2.2	3.3	3.3	3.3
Titration of Complement.	10.0	10.0	5.0	6.6	5.0	10.0	10.0	10.0	6.6	6.6	6.6	10.0	10.0	13.3	13.3	10.0	10.0	10.0	20.0	10.0
Bactericidal reaction with B. coli "X"	2	0	1	1	2	0	1	1	2	0	1	0	1	1	0	3	2	2	1	3
Bactericidal reaction with Str. haemolyticus	1	1	-	1	1	3	3	3	2	2	3	2	3	3	3	2	2	2	0	0
Agglutination reaction with B. abortus (Hog)	8	64	64	64	32	64	64	256	64	256	64	128	128	256	128	256	32	64	128	128

	21/3/32.					23/3/32.					28/3/32.					30/3/32.				
Number.	408.	422.	426.	435.	445.	411.	420.	437.	438.	440.	401.	424.	442.	447.	458.	403.	418.	443.	446.	555.
Haemolytic Reaction.	2.5	3.3	2.8	3.3	3.3	2.0	2.2	2.0	2.8	2.2	3.3	2.8	2.2	2.0	2.5	3.3	2.8	3.3	2.0	3.3
Titration of Complement.	10.0	13.3	13.3	13.3	13.3	4.0	4.0	5.0	4.0	5.0	10.0	13.3	10.0	6.6	6.6	5.0	10.0	10.0	10.0	10.0
Bactericidal reaction with B. coli "X"	3	1	4	2	2	2	3	0	0	0	0	-	-	-	-	2	1	1	1	1
Bactericidal reaction with Str. haemolyticus	4	2	2	3	3	0	0	2	2	0	2	2	3	2	3	-	-	0	-	-
Agglutination reaction with B. abortus (Hog)	256	64	128	64	64	64	32	64	32	64	128	128	128	128	256	128	128	64	128	256

	11/4/32.					13/4/32.				
Number.	399.	432.	444.	450.	453.	577.	580.	583.	584.	590.
Haemolytic Reaction.	3.3	4.0	3.3	3.3	2.5	2.5	3.3	3.5	2.8	2.5
Titration of Complement.	5.0	5.0	6.6	5.0	10.0	5.0	6.6	5.0	4.0	4.0
Bactericidal reaction with B. coli "X"	5	4	0	1	5	2	0	0	4	0
Bactericidal reaction with Str. haemolyticus	2	1	1	1	1	-	0	-	0	0
Agglutination reaction with B. abortus (Hog)	32	256	256	64	64	128	64	32	16	16

GARROCHORAN

HOGGS.

Heft I.

29/10/30.

Number.	4.	25.	27.	41.	42.
Haemolytic Reaction.	2.2	1.5	1.8	1.5	1.8
Bactericidal reaction with *B. suipestifer*	2	2	2	0	2
Bactericidal reaction with *B. coli* "X"	0	0	2	0	2
Bactericidal reaction with *B. coli* "F_3)	0	0	0	0	0
Agglutination reaction with *B. paratyphosus* B.	64	16	64	64	32
Agglutination reaction with *B. abortus* (Hog)	-	-	-	-	-

4/2/31.

Haemolytic Reaction.	2.5	2.8	2.5	2.0	2.8
Bactericidal reaction with *B. suipestifer*	3	2	2	0	2
Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
Bactericidal reaction with *Str. haemolyticus*	-	-	-	-	-
Agglutination reaction with *B. paratyphosus* B.	8	32	16	8	16
Agglutination reaction with *B. abortus* (Hog)	>128	64	128	64	64

8/4/31.

Haemolytic Reaction.	2.8	2.2	2.5	1.8	2.5
Bactericidal reaction with *B. suipestifer*	2	2	2	2	2
Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
Bactericidal reaction with *Str. haemolyticus*	8	4	0	6	4
Agglutination reaction with *B. paratyphosus* B.	32	16	8	64	32
Agglutination reaction with *B. abortus* (Hog)	128	64	8	64	64

4/6/31.

Number.	4.	25.	42.	60.	78.
Haemolytic Reaction.	1.8	1.5	2.2	2.2	1.4
Bactericidal reaction with *B. suipestifer*	5	5	6	4	2
Bactericidal reaction with *B. coli* "X"	0	0	0	0	0
Bactericidal reaction with *Str. haemolyticus*	-	-	-	-	-
Agglutination reaction with *B. paratyphosus* B.	8	8	32	16	4
Agglutination reaction with *B. abortus* (Hog)	64	128	64	128	64

EXPERIMENT.

Heft I.

6/11/30.

1.	3.	34.	60.	78.	
1.5	1.5	1.8	1.5	1.5	
2	2	2	2	2	
0	0	0	2	0	
0	0	0	0	0	
8	4	8	8	8	
128	128	32	64	128	

11/2/31.

2.5	3.3	5.0	3.3	2.0	
4	4	2	2	-	
2	2	2	0	0	
0	0	0	2	0	
64	16	4	4	8	
-	-	-	-	-	

15/4/31.

2.8	2.5	2.8	3.3	2.0	
4	2	4	4	2	
0	0	0	0	0	
0	0	4	0	4	
8	16	8	16	16	
32	64	128	64	64	

8/6/31.

22.	77.	82.	88.	89.	
2.8	1.8	2.5	2.2	1.5	
4	4	5	2	4	
0	2	0	0	0	
0	0	2	2	0	
16	8	8	16	4	
64	128	64	64	64	

Heft II.

29/10/30.

93.	156.	203.	207.	210.
1.8	1.5	1.8	1.5	1.8
2	2	2	2	2
2	0	0	0	0
0	0	0	0	0
128	64	32	16	64
-	-	-	-	-

4/2/31.

2.5	2.5	2.0	2.5	1.8
0	2	2	2	2
0	0	0	0	0
-	-	-	-	-
16	8	8	16	8
32	64	128	32	64

6/4/31.

1.8	1.5	2.5	2.2	1.6
4	4	5	2	4
0	0	0	0	0
4	2	2	0	2
16	4	8	16	16
32	32	64	64	64

4/6/31.

129.	193.	210.	215.	229.
1.8	2.2	1.5	1.5	1.8
5	4	2	5	4
0	0	2	0	0
-	-	-	-	-
8	16	16	8	16
128	64	8	32	128

Heft III.

6/11/30.					29/10/30.				
108.	111.	186.	193.	205.	267.	273.	284.	307.	310.
1.5	1.8	1.8	1.8	1.8	1.5	1.8	2.2	1.5	1.8
2	2	2	4	2	2	2	2	2	2
0	2	2	0	2	0	0	0	0	2
0	0	0	0	-	0	0	0	0	0
8	8	4	16	4	32	16	32	32	32
64	128	128	128	64	-	-	-	-	-
11/2/31.					4/2/31.				
2.2	1.5	2.8	2.8	1.8	2.8	2.2	2.5	1.8	2.5
-	4	2	2	2	2	0	2	2	2
0	0	2	0	2	0	0	0	0	0
0	0	0	0	0	-	-	-	-	-
16	16	32	32	32	16	8	16	4	8
-	-	-	-	-	32	64	64	32	64
13/4/31.					6/4/31.				
103.	108.	186.	193.	205.	267.	284.	297.	307.	310.
3.3	3.3	2.8	3.3	2.8	2.5	2.5	2.5	2.2	2.8
4	4	-	-	4	4	4	2	4	4
2	0	0	0	0	2	0	0	0	0
2	0	0	0	0	0	2	0	0	0
8	16	16	8	8	8	16	16	4	8
> 256	128	128	>256	64	64	64	64	64	64
8/6/31.					4/6/31.				
95.	110.	171.	213.	224.	267.	281.	295.	297.	317.
1.8	2.2	3.3	2.8	2.8	2.2	1.8	2.2	2.0	1.8
4	4	4	4	4	4	4	6	6	4
2	0	0	0	0	2	2	0	0	0
0	0	0	0	0	-	-	-	-	-
8	16	16	8	8	8	8	4	<4	4
64	64	64	64	32	32	32	64	64	64

Heft IV.

6/11/30.					29/10/30.				
257.	265.	314.	378.	384.	394.	419.	433.	440.	458.
1.5	2.2	1.5	1.8	1.3	1.5	2.2	1.5	1.8	1.3
2	4	0	2	2	2	2	2	2	2
0	2	0	0	2	0	2	0	2	2
0	0	0	0	0	0	0	0	0	0
8	16	8	16	16	128	64	64	32	16
64	32	64	32	64	-	-	-	-	-
11/2/31.					4/2/31.				
4.0	1.5	3.3	2.2	1.5	-	2.5	2.2	2.5	2.0
4	2	2	4	2	-	2	2	2	2
0	0	2	2	2	-	0	0	0	0
0	0	0	0	0	-	0	0	0	0
64	64	16	16	16	-	16	16	8	4
-	-	-	-	-	-	64	64	64	64
15/4/31.					8/4/31.				
3.3	2.5	2.5	2.8	2.5	-	4.0	2.8	2.8	1.5
2	2	4	4	4	-	2	2	0	2
0	0	0	0	0	-	0	0	0	0
-	2	0	2	0	-	0	2	2	0
8	8	16	8	8	-	16	8	32	8
64	64	32	64	128	-	8	<8	128	16
8/6/31.					4/6/31.				
282.	291.	337.	339.	378.	408.	421.	433.	440.	458.
2.2	2.8	2.8	2.0	2.0	2.5	1.8	2.2	1.8	1.5
4	4	4	0	4	4	4	4	4	5
0	0	2	0	0	0	0	0	0	0
0	2	0	-	2	-	-	-	-	-
16	8	8	8	16	8	16	8	4	16
32	64	64	64	64	32	256	32	64	32

6/11/30.					
393.	408.	421.	423.	583.	
1.8	1.5	1.5	1.5	1.8	
0	0	4	2	0	
0	0	0	0	0	
0	0	0	0	0	
8	8	32	4	16	
32	64	128	64	128	
11/2/31.					
1.8	2.8	3.3	2.5	2.8	
2	4	4	2	2	
0	2	0	2	0	
0	0	0	0	0	
32	64	16	32	8	
-	-	-	-	-	
13/4/31.					
2.8	2.2	2.8	2.2	1.8	
-	4	5	4	4	
0	0	0	0	0	
0	0	2	0	0	
8	32	32	16	16	
128	128	128	32	128	
8/6/31.					
395.	411.	418.	455.		
2.2	1.5	2.2	2.2		
4	4	4	4		
2	0	0	0		
0	0	2	0		
8	16	16	16		
128	16	64	64		

GARROCHORAN.

GIMMERS.

EXPERIMENT.

Heft I.

27/10/30.	Number.	484.	486.	490.	495.
Haemolytic Reaction.		1.8	1.8	1.6	1.8
Bactericidal reaction with	B. suipestifer	5	4	5	4
	B. coli "X"	0	0	0	0
	B. coli "F_3"	0	0	0	2
Agglutination reaction with	B. paratyphosus B.	64	32	64	64
	B. abortus (Hog)	-	-	-	-
2/2/31.					
Haemolytic Reaction.		1.2	1.6	1.3	1.5
Bactericidal reaction with	B. suipestifer	4	4	4	2
	B. coli "X"	2	0	0	2
	Str. haemolyticus	0	0	0	0
Agglutination reaction with	B. paratyphosus B.	32	16	32	64
	B. abortus (Hog)	8	4	64	4
8/4/31 & 6/4/31.					
Haemolytic Reaction.		1.8	2.5	2.2	2.2
Bactericidal reaction with	B. suipestifer	2	2	2	2
	B. coli "X"	0	0	0	0
	Str. haemolyticus	0	0	0	0
Agglutination reaction with	B. paratyphosus B.	64	32	32	16
	B. abortus (Hog)	8	128	64	128

2/6/31.	Number.	473.	475.	480.	488.	490.
Haemolytic Reaction.		2.5	3.3	1.6	1.8	1.8
Bactericidal reaction with	B. suipestifer	4	4	4	4	4
	B. coli "X"	0	0	0	0	0
	Str. haemolyticus	0	2	-	0	-
Agglutination reaction with	B. paratyphosus B.	16	32	32	32	32
	B. abortus (Hog)	64	64	64	128	64

NOTE:- Hefts I & IV were sampled on 8/4/31 and Hefts II & II on 6/4/31.

Heft II.

499.	508.	524.	529.	539.	547.	550.	556.	560.	573.
1.8	1.5	1.8	2.5	1.8	1.8	2.2	2.0	2.2	2.2
4	2	4	4	4	4	5	4	5	4
0	0	0	0	0	0	0	0	0	0
2	0	2	2	2	2	0	0	2	0
32	32	128	32	64	64	64	64	64	32
-	-	-	-	-	-	-	-	-	-
2.2	1.5	2.2	2.2	1.3	1.8	1.8	1.3	1.3	1.2
4	4	4	4	4	2	5	4	4	5
2	0	2	2	2	2	0	0	0	0
0	0	0	0	0	0	0	2	0	0
16	32	32	8	32	32	32	16	16	16
32	16	32	8	4	4	16	128	4	16
2.8	2.2	1.8	2.8	1.5	5.0	2.2	2.2	3.3	2.2
4	4	4	4	4	4	4	4	5	4
2	0	0	0	0	0	0	0	0	0
0	0	0	2	0	0	2	0	-	-
8	16	32	64	16	32	8	8	32	8
32	16	32	64	64	16	32	64	64	32

499.	501.	509.	522.	529.	546.	563.	565.	571.	573.
1.3	1.3	2.5	1.8	1.8	1.5	1.6	1.8	1.5	1.6
5	4	4	4	4	4	5	4	4	4
0	0	0	0	0	0	0	0	0	0
-	-	-	0	0	0	0	0	-	-
8	16	32	16	16	16	32	32	32	16
64	128	64	64	128	64	64	128	128	64

Heft III.

Heft IV.

576.	577.	580.	585.	586.
2.2	2.2	1.8	1.5	1.4
4	4	4	4	4
0	0	0	0	0
0	0	2	2	0
32	32	64	64	64
-	-	-	-	-
1.2	1.8	1.3	1.5	1.3
4	4	4	4	4
2	0	0	2	0
0	0	2	0	0
16	16	16	64	32
32	8	8	4	64
2.5	1.8	2.2	1.8	1.8	591. 1.8
2	2	2	2	2	2
0	0	0	0	0	0
0	2	4	2	4	2
16	8	16	32	32	128
8	8	64	16	64	8
583.	584.	585.	590.	591.	
2.2	1.8	1.5	1.5	1.5	
4	4	4	4	4	
0	0	0	0	0	
0	2	0	0	0	
32	-	-	-	-	
32	32	64	64	128	

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BARREN

Heft I.

3/11/30.

Number.	11.	16.	48.	54.	58.
Haemolytic Reaction.	-	2.0	1.8	1.8	1.8
Bactericidal reaction with B. suipestifer	2	3	3	3	2
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with B. coli "F_3"	0	0	0	0	-
Agglutination reaction with B. paratyphosus B.	16	8	32	8	16
Agglutination reaction with B. abortus (Hog)	16	32	>256	64	>256

9/2/31.

Number.	11.	16.	48.	54.	58.
Haemolytic Reaction.	3.3	4.0	5.0	4.0	2.8
Bactericidal reaction with B. suipestifer	4	4	4	2	4
Bactericidal reaction with B. coli "X"	2	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	16	64	32	32
Agglutination reaction with B. abortus (Hog)	128	256	256	256	256

15/4/31.

Number.	11.	16.	48.	54.	58.
Haemolytic Reaction.	3.3	4.0	2.8	3.3	2.2
Bactericidal reaction with B. suipestifer	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	4	0	4	4
Agglutination reaction with B. paratyphosus B.	8	16	32	8	32
Agglutination reaction with B. abortus (Hog)	64	64	64	128	128

EXPERIMENT.

EWES.

Heft II.

3/11/30.

	105.	106.	136.	153.	215.
Haemolytic Reaction.	1.6	2.2	2.0	2.2	2.0
Bactericidal reaction with B. suipestifer	2	2	2	2	2
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	16	4	8	16
Agglutination reaction with B. abortus (Hog)	16	>256	32	2	64

9/2/31.

	105.	106.	136.	153.	215.
Haemolytic Reaction.	2.5	3.3	2.8	3.3	2.8
Bactericidal reaction with B. suipestifer	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	2	2	2
Bactericidal reaction with Str. haemolyticus	0	0	0	2	4
Agglutination reaction with B. paratyphosus B.	32	16	16	8	4
Agglutination reaction with B. abortus (Hog)	128	128	128	64	256

13/4/31.

	105.	106.	133.	153.	285.
Haemolytic Reaction.	2.8	4.0	1.8	2.8	2.2
Bactericidal reaction with B. suipestifer	4	4	2	4	4
Bactericidal reaction with B. coli "X"	0	2	0	2	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	16	32	8	16
Agglutination reaction with B. abortus (Hog)	128	>256	128	128	256

Heft III.

3/11/30.

	269.	303.	335.	371.	376.
Haemolytic Reaction.	1.8	1.8	1.8	2.2	2.2
Bactericidal reaction with B. suipestifer	3	2	2	3	-
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	8	16	32	32
Agglutination reaction with B. abortus (Hog)	16	8	16	32	64

9/2/31.

	269.	289.	303.	371.	376.
Haemolytic Reaction.	2.2	2.8	4.0	3.3	5.0
Bactericidal reaction with B. suipestifer	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	2	2
Bactericidal reaction with Str. haemolyticus	2	0	0	0	0
Agglutination reaction with B. paratyphosus B.	8	16	16	8	16
Agglutination reaction with B. abortus (Hog)	>256	>256	128	256	128

15/4/31.

	269.	303.	335.	371.	376.
Haemolytic Reaction.	2.8	2.8	2.2	2.8	2.8
Bactericidal reaction with B. suipestifer	4	4	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	2	0
Agglutination reaction with B. paratyphosus B.	16	32	16	8	8
Agglutination reaction with B. abortus (Hog)	64	32	64	128	64

Heft IV.

3/11/30.

	396.	423.	426.	434.	443.
Haemolytic Reaction.	2.2	2.2	1.8	2.0	2.2
Bactericidal reaction with B. suipestifer	3	3	3	3	2
Bactericidal reaction with B. coli "X"	0	-	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	32	64	8	16	16
Agglutination reaction with B. abortus (Hog)	>256	16	64	32	32

9/2/31.

	396.	423.	426.	434.	443.
Haemolytic Reaction.	1.8	5.0	2.8	3.3	4.0
Bactericidal reaction with B. suipestifer	4	2	4	4	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	0
Agglutination reaction with B. paratyphosus B.	16	8	16	16	16
Agglutination reaction with B. abortus (Hog)	>256	128	>256	128	128

13/4/31.

	396.	423.	426.	434.	443.
Haemolytic Reaction.	2.2	1.8	2.2	2.2	2.5
Bactericidal reaction with B. suipestifer	4	5	4	2	4
Bactericidal reaction with B. coli "X"	0	0	0	0	0
Bactericidal reaction with Str. haemolyticus	0	0	0	0	2
Agglutination reaction with B. paratyphosus B.	8	16	32	32	8
Agglutination reaction with B. abortus (Hog)	128	256	128	>256	64

Gal Pochoran -

Ratio $\frac{\text{range of means}}{\text{mean of ranges}}$ for each reaction on each day, with corresponding values of P, where calculated (by the analysis of variance method).

The ratios are arranged in descending order of magnitude

Haemolytic Reaction.

Date.	Ratio.	P.
9/11/31.	1.50	<0.01
21/5/30.	0.96	
2/11/31.	0.94	0.01-0.05
25/3/31.	0.92	0.01-0.05
23/3/32.	0.92	
27/5/31.	0.88	0.01-0.05
29/5/31.	0.78	>0.05
22/10/30.	0.77	>0.05
21/3/32.	0.73	
23/3/31.	0.71	>0.05
19/1/31.	0.66	>0.05
17/11/31.	0.60	>0.05
24/11/31.	0.56	
23/5/31.	0.54	
28/1/31.	0.53	>0.05
26/1/31.	0.48	>0.05
21/5/31.	0.46	
28/3/31.	0.46	
26/11/31.	0.41	
25/10/30.	0.39	
16/11/31.	0.39	
13/10/30.	0.36	
21/1/31.	0.36	>0.05
5/11/31.	0.35	
13/4/32.	0.30	
30/3/32.	0.26	
15/7/30.	0.24	>0.05
11/4/32.	0.22	

Titration of Complement.

Date.	Ratio.	P.
23/11/31.	1.26	<0.01
5/11/31.	0.88	0.01-0.05
25/11/31.	0.74	>0.05
17/11/31.	0.73	
11/4/32.	0.69	
30/3/32.	0.61	
2/11/31.	0.58	
21/3/32.	0.50	
18/11/31.	0.45	
23/3/32.	0.40	
13/4/32.	0.37	
22/7/31.	0.25	
19/11/31.	0.22	
20/7/31.	0.18	

Bactericidal with B. suipestifer.

Date.	Ratio.	P.
23/3/31.	1.2	0.05
19/1/31.	1.0	0.01-0.05
22/10/30.	0.8	>0.05
28/1/31.	0.8	
23/5/32.	0.8	
20/7/31.	0.8	
22/7/31.	0.8	
29/5/31.	0.8	
21/1/31.	0.64	
15/10/30.	0.6	
26/1/31.	0.4	
25/3/31.	0.4	
21/5/31.	0.4	
27/5/31.	0.4	
13/10/30.	0.34	

(see text p.92.)

Bactericidal Reaction. with B. coli "X"

Date.	Ratio.	P.
30/3/32.	1.03	>0.05
21/1/31.	0.8	>0.05
28/1/31.	0.8	
21/5/31.	0.8	
29/5/31.	0.8	
20/7/31.	0.8	
22/7/31.	0.8	
18/11/31.	0.8	
30/3/32.	0.8	
5/11/31.	0.66	
11/4/32.	0.64	
25/11/31.	0.6	
11/11/31.	0.53	
17/11/31.	0.5	
27/5/31.	0.4	
2/11/31.	0.4	
13/4/32.	0.4	
21/3/32.	0.36	
23/3/32.	0.36	
23/11/31.	0.32	
18/11/31.	0.23	
23/5/32.	0.20	

Bactericidal with Str.

Date.	Ratio.
28/1/31.	2.4
21/1/31.	1.6
23/11/31.	1.6
30/3/32.	1.4
11/11/31.	1.12
18/11/31.	0.96
19/1/31.	0.8
25/3/31.	0.8
23/5/31.	0.8
22/7/31.	0.8
13/4/32.	0.7
28/3/32.	0.64
25/11/31.	0.6
23/3/32.	0.6
21/3/32.	0.56
9/11/31.	0.46
5/11/31.	0.35
17/11/31.	0.26
11/4/32.	0.26
20/7/31.	0.2

Reaction haemolyticus.

P.
*

Agglutination Reaction with B.paratyphosus B.

Date.	Ratio.	P.
22/10/30.	0.9	>0.05
19/1/31.	0.68	
23/3/31.	0.06	
13/10/30.	0.6	
27/3/31.	0.6	
22/7/31.	0.6	
21/1/31.	0.5	
25/3/31.	0.5	
23/5/31.	0.46	
15/10/30.	0.44	
21/5/31.	0.4	
20/7/31.	0.4	
26/1/31.	0.34	
28/1/31.	0.3	
29/5/31.	0.1	

Agglutination Reaction with B.abortus(Hog).

Date.	Ratio.	P.
21/5/31.	1.8	0.01-0.05
5/11/31.	1.06	<0.01
29/5/31.	0.93	0.01-0.05
13/4/32.	0.93	
17/11/31.	0.8	>0.05
18/11/31.	0.8	
21/3/32.	0.8	
30/3/32.	0.8	
28/1/31.	0.75	
23/3/32.	0.62	
22/7/31.	0.66	
24/11/31.	0.64	
2/11/31.	0.57	
23/3/31.	0.53	
26/1/31.	0.5	
23/5/31.	0.46	
11/4/32.	0.44	
17/11/31.	0.4	
28/3/32.	0.4	
9/11/31.	0.36	
27/5/31.	0.32	
21/1/31.	0.3	
13/10/30.	0.27	
20/7/31.	0.25	
15/10/30.	0.23	
22/10/30.	0.23	
25/3/31.	0.20	
26/11/31.	0.20	

* As there was no evidence of any heft maintaining a consistent position relative to the others the values of P were not calculated for this reaction.

Garrochoran - Hoggs, Gimmers and Barren Ewes.

Ratio $\frac{\text{range of means}}{\text{mean of ranges}}$ calculated for each reaction on each day.

The ratios are arranged in descending order of magnitude (see text p.92)

Haemolytic Reaction.			Bactericidal Reaction with *B. suipestifer*.			Bactericidal Reaction with *B. coli* "X".			Agglutination Reaction with *B.paratyphosus B.*			*B.abortus* (Hog) with *Str. haemolyticus*					
Ratio.	Date.	Type.	Ratio.	Date.	Type.	Ratio.	Date.	Type.	Ratio.	Date.	Type.	Ratio.	Date.	Type.	Ratio.	Date.	Type.
1.33	3/11/30.	B.E.	1.1	29/10/30.	G.	0.8	6/11/30.	H.	1.0	2/6/31.	G.	0.83	4/2/31.	H.	0.8	9/2/31.	B.
0.90	2/2/31.	G.	1.07	3/11/30.	B.E.	0.8	9/2/31.	B.E.	0.82	11/2/31.	H.	0.67	6/11/30.	H.	0.8	11/2/31.	H
0.67	27/10/30.	G.	0.8	29/10/30.	H.	0.8	4/6/31.	H.	0.70	9/2/31.	B.E.	0.59	9/2/31.	B.E.	0.4	2/2/31.	G.
0.63	2/6/31.	G.	0.58	11/2/31.	H.	0.6	2/2/31.	G.	0.70	4/6/31.	H.	0.50	2/6/31.	G.	0.4	2/6/31.	G.
0.54	8/6/31.	H.	0.52	6/11/30.	H.	0.4	29/10/30.	H.	0.53	6/11/30.	H.	0.40	3/11/30.	B.E.	0.3	8/6/31.	H.
0.48	11/2/31.	H.	0.47	8/6/31.	H.	0.2	11/2/31.	H.	0.47	3/11/30.	B.E.	0.36	4/6/31.	H.			
0.40	9/2/31.	B.E.	0.42	2/2/31.	G.	0.05	8/6/31.	H.	0.42	8/6/31.	H.	0.33	8/6/31.	H.			
0.28	4/6/31.	H.	0.4	9/2/31.	B.E.				0.36	29/10/30.	H.	0.12	2/2/31.	G.			
0.26	4/2/31.	H	0.4	2/6/31.	G.				0.35	2/2/31.	G.						
0.24	6/11/30.	H.	0.36	4/6/31.	H.				0.35	4/2/31.	H.						
0.17	29/10/30.	H.	0.23	4/2/31.	H.				0.16	27/10/30.	G.						

B.E. = Barren Ewes.
H. = Hoggs
G. = Gimmers.

Values of P obtained by the analysis of variance method on comparing all hefts to determine whether any significant differences appeared between them on separate days and during periods of sampling.

Haemolytic Reaction.

Date.	P.
22/10/30.	>0.05
19/1/31.	>0.05
21/1/31.	>0.05
26/1/31.	>0.05
28/1/31.	>0.05
19-28/1/31.	>0.05
23/3/31.	0.05
25/3/31.	0.01-0.05
30/3/31.	<0.01
1/4/31.	<0.01
23/3-1/4/31	<0.01
27/5/31.	0.01-0.05
29/5/31.	>0.05

Titration of Complement.

Date.	P.
5/11/31.	0.01-0.05
26/11/31.	>0.05

Bactericidal reaction with B.suipestifer.

Date.	P.
13/10/30.	>0.05
19/1/31.	0.01-0.05
23/3/31.	0.05

Bactericidal reaction with B.coli "X".

Date.	P.
20/7/31.	>0.05
28/3/32.	>0.05

Agglutination reaction with B.paratyphosus B.

Date.	P.
22/10/30	>0.05

Agglutination reaction with B.abortus(Hog)

Date.	P.
23/3/31.	0.01-0.05
25/3/31.	0.01-0.05
23 & 25/3/31.	0.01-0.05
23/5/31.	0.01-0.05
27/5/31.	>0.05
29/5/31.	0.01-0.05
23-29/5/31.	>0.05
5/11/31.	<0.01
17/11/31	>0.05
18/11/31	>0.05

Values of P obtained on comparing results from period to period by Fisher's method for testing the significance of means.

Heft	I.	II.	III.	IV.	I-IV.
Ewes.					
Haemolytic Reaction.					
October & January.	0.2	0.05	0.2	0.02	0.5
January & March.	0.7	0	0.3-0.2	0.8-0.7	0.7
October & March.	0.2	0.02	0.5	<0.01	
March & May.	0.01	<0.01	0.1	<0.01	0.05-0.02
May & July.	0.9	0.4-0.3	0.8-0.7	0.3	>0.9
Titration of Complement.					
July & November.		<0.01	<0.01	<0.01	
November & March.	0.2	0.8-0.7	0.6-0.5	>0.9	
Bactericidal Reaction with B. suipestifer.					
October & January.	>0.2	>0.2			>0.5
January & March.	>0.8-0.7	>0.3			insig.
March & May.	>0.8-0.7	>0.7			>0.4
Bactericidal Reaction with B. coli "X"					
March & May.					>0.4
Agglutination Reaction with B. paratyphosus B.					
October & January.					0.8-0.7
January & March.					>0.9
March & May.					0.8
May & July.					0.2-0.1
Agglutination Reaction with B.abortus (Hog)					
October & January.					0.5-0.4
January & March.					0.2-0.1
March & May					0.4
May & July.					0.9
Hoggs.					
Haemolytic Reaction.					
October & January.	0.1-0.05	0.02	0.02	0.05-0.02	<0.01
January & April.	0.4	0.6-0.5	0.7-0.6	>0.9	
April & June.	0.5-0.4	0.7-0.6	0.4	0.5-0.4	

Values of P obtained on comparing the results of cutaneous toxin tests in various hefts. ($P\chi^2$) method.

I. Ewe hoggs - 30th May, 1931. (3 in each heft)

Heft.	I.	II.	III.	IV.
I.	1	1	0.27	0.08
II.		1	0.27	0.08
III.			1	0.014
IV.				1

Hefts III & IV differ significantly from one another.
The order of the hefts is III >I & II >IV.

II. Sheep of varying ages - 6th June, 1931.(18-20 in each heft).

Heft.	I.	II.	III.	IV.
I.	1	0.24	0.07	0.114
II.		1	0.39	0.008
III.			1	0.0025
IV.				1

Hefts III & IV differ significantly, also II & IV.
The order of the hefts is III >II >I >IV.

III. Hoggs and Gimmers - 27th June, 1931. (9-13 in each heft)

Heft.	I.	II.	III.	IV.
I.	1	1	0.89	0.58
II.		1	0.90	0.60
III.			1	0.66
IV.				1

No significant differences.

IV. Gimmers only - 6th & 27th June, 1931. (11-7 in each heft)

Heft.	I.	II.	III.	IV.
I.	1	0.41	0.18	0.47
II.		1	0.41	0.13
III.			1	0.07
IV.				1

There are no significant differences, though that between hefts III & IV approaches significance.

V. Hoggs only - 30th May, 6th & 27th June, 1931. (12 in each heft)

No significant differences.

VI. All Sheep tested on 30th May, 6th & 27th June, 1931.(32-34 in each heft)

Heft.	I.	II.	III.	IV.
I.	1	0.38	0.18	0.16
II.		1	0.65	0.026
III.			1	0.0075
IV.				1

Hefts III and IV differ significantly, also II and IV.
The order of the hefts is III> II> I> IV.

VII. Sheep 1½ years old - 4th October, 1931.(many previously inoculated)
(15 in each heft).

Heft.	I.	II.	III.	IV.
I.	1	0.20	1	0.41
II.		1	0.20	0.62
III.			1	0.41
IV.				1

No significant difference.

Numerical Example of Application of Analysis of Variance Method. (see p.92)

Haemolytic Reaction. 23rd March, 1931.

Heft.	x M.H.D.s per c.c.	$\bar{x}_p$.	$x-\bar{x}$.	$(100\ (x-\bar{x}))^2$	$x-\bar{x}_p$.	$(100\ (x-\bar{x}_p))^2$	$\bar{x}_p-\bar{x}$.	$(100\ (\bar{x}_p-\bar{x}))^2$
	1.53		-0.26	676	-0.25	625		
	1.53		-0.26	676	-0.25	625		
I	1.81	1.78	0.02	4	0.03	9	-0.01	1
	1.81		0.02	4	0.03	9		
	2.20		0.41	1681	0.42	1764		
	1.33		-0.46	2116	-0.38	1444		
	1.81		0.02	4	0.10	100		
II.	1.81	1.71	0.02	4	0.10	100	-0.08	64
	1.81		0.02	4	0.10	100		
	1.81		0.02	4	0.10	100		
	1.33		-0.46	2116	-0.76	5776		
	2.20		0.41	1681	0.11	121		
III.	2.20	2.09	0.41	1681	0.11	121	0.30	900
	2.20		0.41	1681	0.11	121		
	2.50		0.71	5041	0.41	1681		
	1.33		-0.46	2116	-0.26	676		
	1.33		-0.46	2116	-0.26	676		
IV.	1.66	1.59	-0.13	196	0.07	49	-0.20	400
	1.81		0.02	4	0.22	484		
	1.81		0.02	4	0.22	484		
$\bar{x}$ = 1.79.				Total 21809 = X.		15066 = Y		1365 = $\frac{Z}{5}$

$$X - Y = Z.$$

Replacing these with corresponding values from the preceding table 21809 - 15066 6825.

$$\frac{Y}{16} = 942 = b \text{ (the variance corresponding to 15 degrees of freedom due to individual variation factor).}$$

$$\frac{Z}{3} = 2275 = a \text{ (the variance corresponding to 3 degrees of freedom due to diet factor).}$$

$$z = \tfrac{1}{2}(\log_e 2275 - \log_e 942).$$
$$= 0.5405.$$

The value of P corresponding to z = 0.5876 is 0.05 entering the table with n_1 = and n_2 =

The differences between the diets on this day were therefore not significant.

z was calculated for 25th March in the same way and was found to be 0.6671.

The value of P corresponding to z = 0.5876 is 0.05 and to z = 0.8331 is 0.01 entering the table as above.

∴ The value of P corresponding to z = 0.6671 lies between 0.05 and 0.01.

Taking the two days together

$$Y_1 \quad Y_2 = 31468 = P.$$
$$Z_1 \quad Z_2 = 11330 = Q.$$

$$A = \frac{11330}{6} = \text{variance corresponding to 6 degrees of freedom due to diet factor.}$$

$$B = \frac{31468}{32} = \text{variance corresponding to 32 degrees of freedom due to individual variation factor.}$$

$$z = \tfrac{1}{2}(\log_e A - \log_e B).$$
$$= 0.5617.$$

This value of z lies between those corresponding to P = 0.01 and P = 0.05, which are 0.6226 and 0.4220 respectively and the dietary factor thus causes a signific-:ant difference when the two days are considered together.